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Editorial Views and News

Every year, the International Association for Dental Research co-sponsors the program of the Nd Section (Dentistry) of the American Association for the Advancement of Science. This significant Symposium is always held in the final week of the year and has become extremely well attended by researchers from all parts of the United States as well as from nearby lands.

This year the Symposium on Dental Genetics has been arranged by Albert A. Dahlberg, of the University of Chicago, who is Program Committee Chairman for 1961. Other Nd Section officers are Harold J. Noyes, Vice-Chairman; Reidar F. Sognnaes, Secretary; and Beryl T. Ritchey, in charge of Local Arrangements.

1961 A.A.A.S. (Nd) PROGRAM

WEDNESDAY, DECEMBER 27, DENVER, COLORADO
SILVER GLADE ROOM OF THE COSMOPOLITAN HOTEL

Morning Session, 9:00–12:00 A.M.

1. "Recent Advances in Dental Genetics"
   CARL J. WITKOP, JR.
   Chief, Human Genetics Section, National Institute of Dental Research
   Bethesda, Maryland
2. "The Respective Role of Twin, Sibling, Family, and Population Methods in Dento-medical Studies"
   RICHARD H. OSBORNE
   Division of Preventive Medicine, Sloan-Kettering Institute for Cancer Research, and Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College
   New York, New York
3. "Effects of Heredity and Environment on the Development of the Dentition"
   JEROME D. NISWANDER
   Department of Human Genetics, University of Michigan Medical School
   Ann Arbor, Michigan
4. "Chromosome Nondisjunctions and Oral Anomalies"
   ROBERT J. GORLIN
   Department of Oral Pathology, University of Minnesota
   Minneapolis, Minnesota
5. "The Effectiveness of Selection in Producing Laboratory Stocks Genetically Uniform for Resistance or Susceptibility to Dental Caries"
   HARRISON R. HUNT
   Professor Emeritus of Zoology, Michigan State University
   East Lansing, Michigan

   and

   SAMUEL ROSEN
   School of Dentistry, Ohio State University
   Columbus, Ohio

Noon, Special Luncheon in adjacent room

Afternoon Session, 2:00–4:30 P.M.

6. "Family Studies of the Facial Complex"
   BERTRAM HANNA
   Human Genetics Section, National Institute of Dental Research
   Bethesda, Maryland
7. "Some Clinical Aspects of Genetic Research in Dentistry"
   SIDNEY L. HOROWITZ
   Institute of Reconstructive Plastic Surgery, New York University, Bellevue Medical Center, and the School of Dental and Oral Surgery, Columbia University
   New York, New York
8. "Third Molar Polymorphism and Dental Genetics"
   STANLEY M. GARN and A. B. LEWIS
   Department of Growth and Genetics, The Fels Research Institute
   Yellow Springs, Ohio
9. "The Regulative Changes in Tooth Germs Grown in Tissue Culture"
   SHIRLEY GLASSTONE HUGHES
   Strangeways Research Laboratories, Wort's Causeway
   Cambridge, England

Round Table Discussion, 4:30 P.M.

Participants in the Symposium will verbalize their informal thinking during the final one and one-half hours of the session.

It is appropriate editorially to make mention of the fact that the United States Public Health Service participated in the support of this program.

Also of direct interest is the other closely related Symposium provided by Section N (Medicine) entitled "Physiological and Biochemical Aspects of Human Genetics." This symposium, arranged by Alexander G. Bearn, of The Rockefeller Institute, and Oscar Touster, of Vanderbilt University, will be held at the A.A.A.S. meeting on December 29 and 30. The program for the two days includes these sections:

I. Structure and Specific Action of DNA
II. Gene-Protein Relationships, Part 1
III. Gene-Protein Relationships, Part 2
IV. Tissue Culture, Immunological and Evolutionary Aspects

It is the editor's hope that the great contributions presented at these symposia will be published as an A.A.A.S. monograph.

FRANK J. ORLAND
Editor

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

FORTIETH ANNUAL MEETING, MARCH 15, 16, 17, AND 18, 1962
SHERATON JEFFERSON HOTEL, ST. LOUIS, MISSOURI

Abstracts of papers for presentation at the Fortieth Annual Meeting of the I.A.D.R. will have to be submitted according to a prescribed format which will be furnished to all members, without solicitation, in early fall of 1961. Non-members may request these instructions from the Assistant Secretary, Mrs. Mildred Erickson, American Institute of Biological Sciences, 2000 P Street, N.W., Washington 6, D.C. Non-members who desire to present papers should be sponsored by a member. Abstracts will be limited to 250 words and must have substantial material in them to indicate that results have been obtained. The final date for receipt of abstracts will be December 1, 1961. When the instructions are received, they should be read carefully to prevent rejection of submitted material.

Abstracts entered in the Edward H. Hatton Award Competition should conform to the specifications published in the *Journal of Dental Research,* **38**:427, 1959, using the same format as provided with instructions.

Fluorine Content of Superficial Enamel Layer and Its Correlation with the Fluorine Content of Saliva, Tooth Age, and DMFT Count

ITZHAC GEDALIA, KURT A. ROSENZWEIG, and ABRAHAM SADEH

Department of Preventive Dentistry, Hebrew University–Hadassah School of Dentistry, founded by the Alpha-Omega Fraternity, Jerusalem, Israel

With the increasing use of fluorides for the prevention of dental caries, studies of the fluorine content of teeth have gained much in importance. Research on tooth fluorine has been confined mainly to determination of the fluorine content of either separated enamel and dentin[1–6] or whole-tooth substance.[7] Such studies have shown that the fluorine content of the enamel, the dentin, and whole teeth is proportional to the fluorine content of the drinking water and food[1–3, 5, 6] and inversely proportional to the DMFT count.[4, 7]

Recently, attention has been focused on the fluorine content of the superficial enamel layer. It was found that after the tooth erupts into the oral cavity, the superficial enamel incorporates additional fluorine from drinking water, food, and saliva.[8–14]

No attempt has so far been made to correlate the fluorine content of the superficial layer of the enamel of human teeth with the DMFT count. The present investigation was undertaken to determine whether correlations exist between the fluorine content of the superficial enamel of human teeth and (*a*) the fluorine content of the saliva, (*b*) the age of the erupted teeth, (*c*) the DMFT count.

MATERIALS AND METHODS

From 27 soldiers, aged eighteen to thirty years, 30 permanent teeth, non-carious and carious, in more than half of which the crown was intact, were extracted because of caries or periodontal disease. These persons had been born and reared in regions with water of unknown fluorine content. Sampling of teeth from adults who had been born and reared in a place in Israel with a constant known fluorine content in the water supply was impossible because of mass immigration and consequent changes in place of residence and communal water supplies. Age of the individual teeth was estimated according to Orban[15] by deducting the number of years elapsed at eruption time from the present age of the patient.

Calculus and debris were thoroughly removed from the teeth with a scaler and metal brush. Samples of superficial enamel for fluorine analysis were ground off by means of a rotating diamond wheel. Special care was taken not to include any carious material. The grinding method was applied by us for enamel removal because acid washings may produce uneven etching over the surface of the tooth. In addition, fluorine may remain un-

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dissolved when enamel is treated with acid, although Ca and P enter the acid solution.[12] Before grinding, the distance between opposite tooth surfaces was measured at a suitable position with a micrometer. The surface enamel was ground from the buccal, lingual, and approximal surfaces until the desired uniform depth of 0.05 mm., measured with the micrometer, was obtained over each area. The total thickness of enamel removed from opposite surfaces was not more than 0.1 mm. The occlusal and incisal surfaces were not used.[10] In order to minimize loss of the finely powdered enamel, grinding was carried out in a glass cylinder. The powder which accumulated on the walls of the cylinder was transferred to a crucible, ashed, and weighed. The weight of the enamel from each tooth was between 5 and 10 mg.

Saliva samples of approximately 20 ml. each were collected from the patients after breakfast between 9:00 and 1:00, before the tooth extraction. To stimulate the saliva excretion, a piece of paraffin wax was chewed by the patient. The collection of each saliva sample took approximately 10 minutes. All the dental examinations were carried out by the same investigator with plane mouth mirror, explorer, and reflector. The caries experience of each patient at the time of the examination was expressed by the DMFT count.

Technique of fluorine analysis in enamel and saliva.—The weighed ashed enamel was placed in a fluorine still. The saliva samples were made slightly alkaline with fluorine-free $Ca(OH)_2$ solution, which served also as a fluorine fixative. The samples were then evaporated to dryness and ashed at about 400° C. The ashed samples were also transferred to fluorine stills.

The chlorides were precipitated with Ag_2SO_4. Fluorine was steam-distilled at 135°–138° C. in the presence of H_2SO_4 and SiO_2. In order to avoid the carrying-over of sulfuric acid particles by the steam into the distillate, causing interference with the fluorine determination, a distillation head was attached to the fluorine still.[7] In the concentrated distillate (1 ml.), fluorine was determined photocolorimetrically* by the color-fading reaction of a violet iron salicylate complex as described by Voegtlin and Hodge.[16]

This method was tested by performing 18 determinations of fluorine in a pooled saliva sample from one individual. The results gave a range from 0.08 to 0.14 ppm F between the individual determinations, with a mean of 0.11 ppm F and a S.E. of $\pm$ 0.0015. The results are summarized in Tables 1 and 2.

DISCUSSION

The correlation coefficient of the fluorine content of the superficial layer of the enamel and the fluorine content of the saliva, $r = +0.058$, is not significant. The correlation coefficient between the age of the teeth and the fluorine content of the superficial enamel, $r = +0.378$, is significant (Table 2). These findings are partly in agreement with the results of other investigators.[10, 11, 17, 18] By using electron microscopy,[17] radioactive fluorine,[11, 18] and chemical methods,[10] they have proved that fluorine is incorporated into the superficial layer of human tooth enamel. This incorporation takes place posteruptively from saliva, drinking water, and food and is cumulative with respect to the age of the tooth.[10] The absence of a correlation between the fluorine content of the superficial enamel and the fluorine content of saliva may be explained by our lack of knowledge of the concentration of fluorine in the drinking water. The history of the pre- and post-

* Lumetron model 402-E.

eruptive tooth development from the point of view of the fluorine intake through drinking water was unknown in the group we studied, and variations in the exposure to fluorine in drinking water may have influenced the fluorine content of the superficial layer of the enamel. It seems, therefore, that the age of the tooth plays a determining role in the accumulation of fluorine in the superficial enamel layer.

The relationship between the fluorine content of the superficial enamel and the DMFT count suggested by Brudevold, Gardner, and Smith[9] could not be confirmed by us, $r = +0.135$. One of the reasons may be that the DMFT count in the case of an age group such as that of our patients does not necessarily indicate the real extent of dental caries experience because of the presence of crown- and bridgework. Furthermore it appears that the DMFT count is not a sufficiently accurate estimate of caries experience for the purpose of this investigation.

The lack of correlation between the fluorine content of the saliva and the DMFT count, $r = +0.052$, is in agreement with a previous study of the fluorine content of

TABLE 1

FLUORINE CONTENT OF SUPERFICIAL ENAMEL LAYER AND SALIVA, TOOTH AGE, AND DMFT COUNT

No. of Case	Fluorine in Superficial Enamel Layer (ppm)	Fluorine in Saliva (ppm)	Tooth	Tooth Age (Years)	DMFT Count (Number)
1.....	600	0.14	$\underline{\lfloor 5}$	12	7
2.....	100	0.25	$\underline{\lfloor 6}$	11	15
3.....	400	0.40	$\overline{\lfloor 4}$	8	10
4.....	445	0.18	$\underline{\lfloor 8}$	4	16
5.....	700	0.20	$\overline{\lfloor 8}$	12	10
6.....	370	0.10	$\underline{\lfloor 8}$	9	14
7.....	335	0.40	$\underline{4\rfloor}$	9	9
8.....	245	0.10	$\overline{6\rfloor}$	12	7
9.....	550	0.17	$\overline{\lfloor 6}$	12	6
10.....	230	0.46	$\underline{\lfloor 5}$	7	12
11.....	1,000	0.34	.....	.....	9
12.....	660	0.16	$\underline{\lfloor 5}$	8	8
13.....	250	0.15	$\overline{\lfloor 6}$	11	9
14.....	246	0.13	$\overline{6\rfloor}$	12	9
15.....	945	0.10	$\underline{\lfloor 6}$	19	14
16.....	300	0.20	$\overline{\lfloor 7}$	9	12
17.....	400	0.10	$\underline{6\rfloor}$	13	1
18.....	453	0.06	$\overline{\lfloor 7}$	19	9
19.....	200	0.06	$\underline{8\rfloor}$	6	12
20.....	210	0.05	$\underline{\lfloor 6}$	14	17
21.....	715	0.12	$\overline{\lfloor 6}$	14	12
22*.....	850	.....	$\underline{\lfloor 7}$	20	.....
23*.....	200	0.20	$\underline{\lfloor 8}$	13	14
24.....	620	0.15	$\underline{\lfloor 7}$	10	4
25.....	500	0.04	$\overline{\lfloor 8}$	6	8
26.....	900	0.27	$\underline{8\rfloor}$	8	17
27*.....	850	.....	$\underline{8\rfloor}$	15	.....
28*.....	300	0.10	$\underline{\lfloor 7}$	8	16
29*.....	550	.....	$\underline{8\rfloor}$	6	.....
30*.....	550	0.18	$\overline{8\rfloor}$	6	7
Total..	30	27	.....	29	27
Range.	100–1,000	0.04–0.46	.....	...4–20	1–17
Mean..	486	0.18	.....	11	10.5

* In a number of cases teeth were extracted from the same person.

saliva and the DMFT count in pregnant women.[19] It seems, therefore, that the relatively small variations in the fluorine content of our saliva samples, usually in the range between 0.1 and 0.2 ppm fluorine, were too small to influence the DMFT count appreciably. This is also in agreement with Leimgruber, who maintains that only concentrations of about 1 ppm fluorine in the saliva would be effective in reducing the caries rate.[20]

SUMMARY

The superficial enamel layer of 30 extracted human permanent teeth of different eruption periods and 27 saliva samples were analyzed for fluorine. The fluorine content in the superficial enamel samples varied between 100 and 1,000 ppm and in the saliva samples between 0.04 and 0.46 ppm.

No significant correlations between the fluorine content of the superficial enamel and the fluorine content in saliva, on the one hand, and the dental caries experience, on the other, were established. The correlation between the fluorine content in the superficial enamel and tooth age after eruption was statistically significant.

TABLE 2

STATISTICAL ANALYSIS OF CORRELATIONS

	Correlation Coefficient r	Significance
F superf. enamel–F saliva	+0.058	–
F superf. enamel–tooth age	+0.378	+
F saliva–DMFT count	+0.052	–
F superf. enamel–DMFT count	+0.135	–

The authors wish to express their thanks to Mr. Natan Portuguese for technical assistance in this study. Thanks are also due to the Dental Corps of the Israel Defence Army for co-operation.

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Effects of Hypervitaminosis D on the Periodontium of the Hamster

H. FAHMY, W. E. ROGERS, D. F. MITCHELL, and H. E. BREWER*
Indiana University, School of Dentistry, Indianapolis, Indiana

Administration of excessive amounts of vitamin D has been reported to result in modification of the human oral tissues. Rampant dental caries, malocclusion, thinning of the enamel, dentin, and the alveolar process, and rarefaction of the molar roots have been observed in a four-year-old girl.[1] In an older girl overdosed with vitamin D for many years, the effects noted consisted of atubular or poorly tubular root dentin, areas of resorption repaired by irregular dentin, and pulpal calcifications.[2] Effects of hypervitaminosis D on the oral tissues of several experimental animals also have been described. Decreased incisor growth, the occurrence of pulp stones, hypercementosis, ankylosis due to thickened cementoid, alterations in pulpal dentin, hemorrhage of the periodontal membrane, and atrophy of the enamel organ are among the manifestations which have been observed in rats.[3–6] In addition, decreased dimensions of the jaws, malocclusion, sclerosis of the jaw, and calcified deposits in the periodontal membrane and in the free gingivae have been found in dogs fed large amounts of the vitamin.[7, 8]

Many of these changes are of interest because of their possible relationship to periodontal disease. Valuable insight into the pattern of development of this disease might be obtained, therefore, through a study of oral tissues when both diseases (i.e., hypervitaminosis D and periodontal disease) were present. Periodontal disease can be invoked and measured in the Syrian hamster by means of a soft, high-carbohydrate diet.[9] Since neither hypervitaminosis D nor its possible effects have been reported in this animal, it was necessary, first, to establish a basic description of this condition, as reported here.

EXPERIMENTAL METHODS

Thirty male and 30 female hamsters, approximately 4 months old, were arranged in experimental, pair-fed control, and ad libitum–fed control groups in such a way that sex and litter differences were balanced. A laboratory chow diet† and tap water

This investigation was supported in part by U.S.P.H.S. research grant D-432 from the National Institute of Dental Research, National Institutes of Health.

Presented at the thirty-eighth general meeting of the International Association for Dental Research, March 17–20, 1960, Chicago, Illinois.

From a thesis submitted to the graduate faculty, Indiana University, School of Dentistry, in partial fulfilment of the requirements for the M.S.D. degree.

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* New address: Cairo University, School of Dentistry, Cairo, Egypt, U.A.R.

† Purina Laboratory Chow, micromixed. Ralston Purina Co., St. Louis.

were furnished to the animals. Twice a week the hamsters in the experimental group were injected intraperitoneally with 0.01 cc. of sesame oil containing 5,000 U.S.P. units of vitamin D_2. Sixteen injections were given, making a total of 80,000 U.S.P. units for each hamster. On the day following the sixteenth injection—the fifty-sixth experimental day—the animals were sacrificed, and 3 male and 3 female representatives from the experimental group and like numbers of littermates from the two control groups were examined.

A kidney, femur, submaxillary salivary gland, and mandible were removed from each animal for microscopic study. All tissues were fixed in 10 per cent formalin except the salivary glands, which were fixed in Bouin's solution. Half the mandible and a femur were decalcified in 5 per cent formic acid. All the material for histologic study was imbedded in paraffin, and the sections were stained either in hematoxylin and eosin or with Von Kossa stain. The other femur was removed, cleaned of cartilage and marrow, defatted in acetone, and dried to constant weight. Then it was ashed and reweighed. Blood samples were taken from the heart at the time of sacrifice. Plasma calcium was determined on these samples by means of a colorimetric method.[10]

RESULTS

The hamsters lost appetite and body weight and became sluggish within 2 days on the vitamin D regimen. Seven of the animals responded acutely to the dosages of vitamin D administered and were sacrificed during the latter half of the experimental

TABLE 1

PLASMA CALCIUM AND FEMUR CALCIFICATION IN HAMSTERS GIVEN EXCESSIVE AMOUNTS OF VITAMIN D_2 AND IN PAIR-FED AND AD LIBITUM–FED CONTROLS

	PLASMA CALCIUM (MG PER CENT)		FEMUR (ASH WT./DRY WT.) (PER CENT)	
	Mean	S.D.	Mean	S.D.
Males:				
Experimental	15.1	0.61	62.1	1.71
Pair-fed control	12.4	0.33	65.6	1.43
Ad lib–fed control	12.5	0.44	65.2	0.68
Females:				
Experimental	15.9	0.35	54.4	3.82
Pair-fed control	12.3	0.35	64.4	1.80
Ad lib–fed control	12.2	0.45	66.6	0.99

period. In the pair-fed animals a weight loss occurred that remained parallel to that in the experimental group during the first half of the period. Thus it is probable that the decline in weight of the experimental animals during this period was due to anorexia, which is a common finding in hypervitaminosis D. The loss in weight of 13 surviving experimental animals ranged from 13 to 42 per cent throughout the whole experimental period.

All the experimental animals developed hypercalcemia and had a diminished femur ash content, as shown in Table 1. The probability that chance alone accounted for

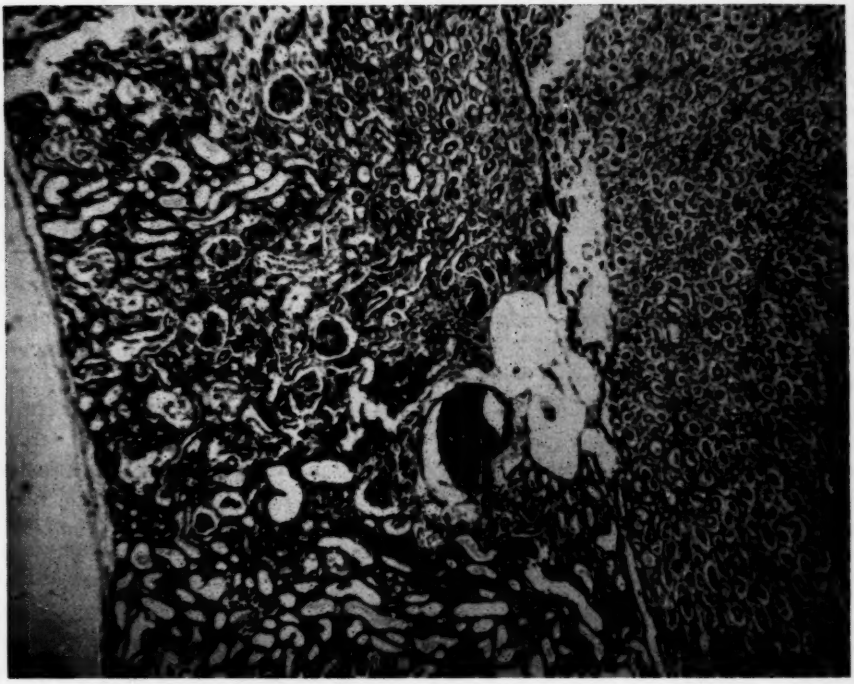

FIG. 1.—Cross-section through kidney of hamster given excess vitamin D. (H and E stain; approximate mag. ×10.)

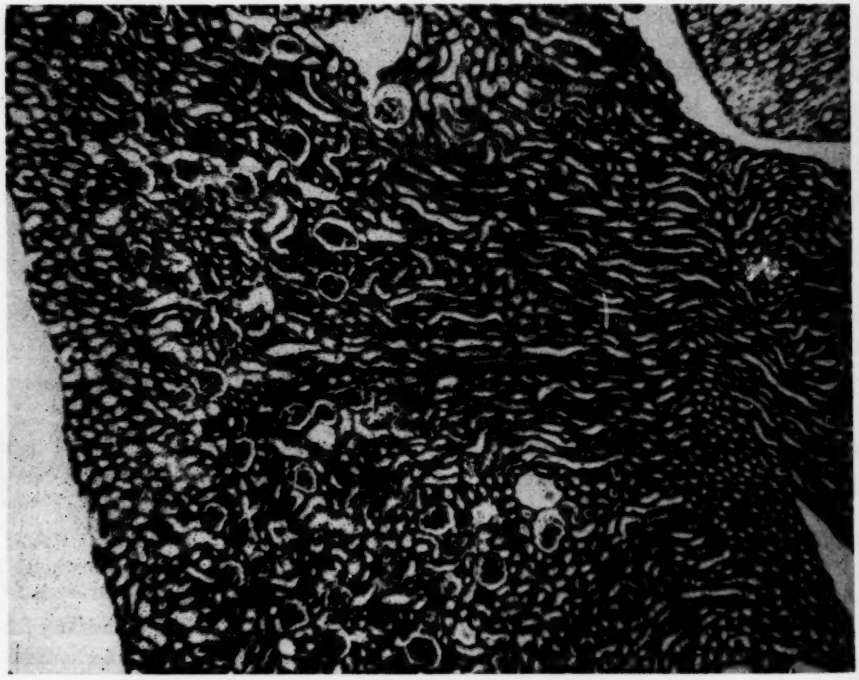

FIG. 2.—Same section as Fig. 1 but of control animal

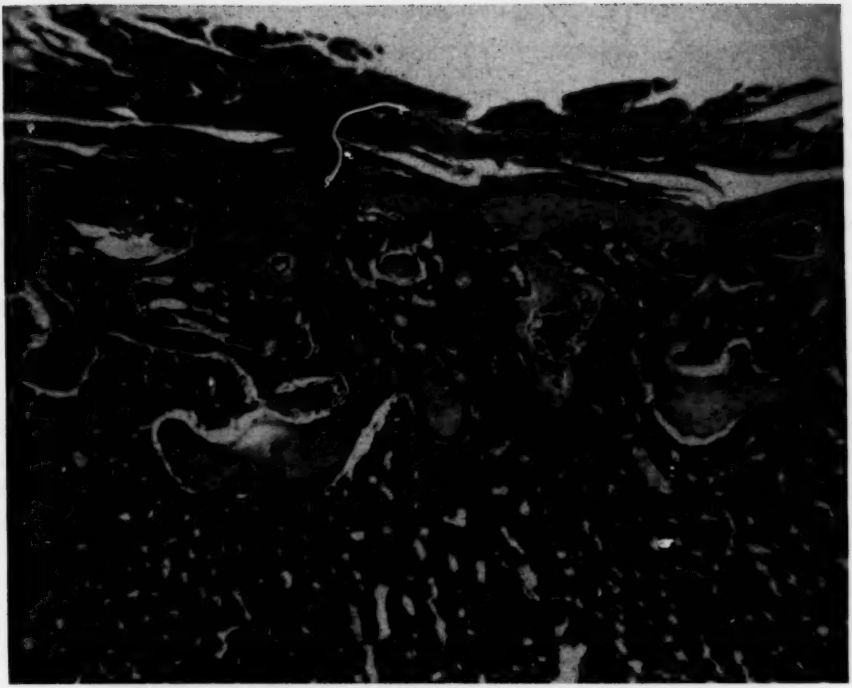

FIG. 3.—Longitudinal section through decalcified femur of hamster given excess of vitamin D. Note thinning of the cortical bone, endosteal resorption, and replacement of the cortex by irregular lamellae with an osteoid lining. (H and E stain; approximate mag. ×20.)

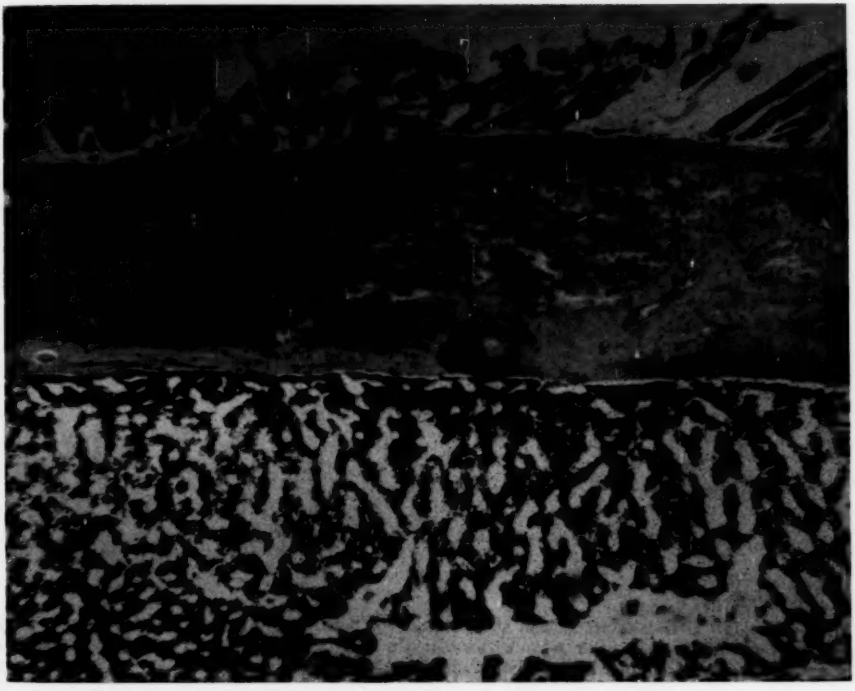

FIG. 4.—Same section as Fig. 3 but of control animal

the differences between the experimental and control groups was always less than 0.01, whereas the difference between the two control groups was statistically insignificant.

Microscopically, varying degrees of vasodilatation and pathologic calcification were seen in the kidneys of the experimental animals. In this organ, pathologic calcification was observed most frequently in the tubules, followed by the blood-vessel walls, interstitial tissue and the glomeruli, and vascular spaces of the cortex. Figure 1 illustrates the kidney involvement in one of the most severely affected animals. The calcifications stained deeply with hematoxylin. Von Kossa–stained sections of the kidneys revealed dense black deposits, further suggesting ectopic calcification. No such lesions were observed in kidneys of the control animals (Fig. 2).

The histologic changes in the femurs of the vitamin D–treated animals were noted in the cortical and marrow portions of the diaphyses only. The principal abnormalities were the numerous excavations on the endosteal aspect of the diaphysis and the irregular outgrowths from the cortex into the marrow cavity (Figs. 3, 4). The excavations were filled with marrow and lined in several places with a pale-staining

FIG. 5.—Mesiodistal decalcified section showing the interdental area between the left mandibular first and second molars of hamster given excess vitamin D. Note calcification of principal membrane fibers, and resorption of alveolar bone and osteoid. (H and E stain; approximate mag. ×35.)

osteoid, indicative of extensive endosteal bone resorption coupled with osteoid deposition. The over-all thickness of the shaft was decreased. In addition, the marrow of the experimental animals appeared more cellular and less fatty than in the controls.

Microscopically, no difference was detected between the submaxillary glands of the treated and untreated hamsters. However, in the molar region the periodontium of the treated animals exhibited central resorption of the interdental and interradicular alveolar bone. Irregular marrow-filled spaces were observed. These spaces occasionally exhibited considerable osteoid formation. The same changes were seen in the central portion of the mandible. The bone between the marrow-filled spaces and the lamina dura presented basophilic lines of pagetoid appearance, suggestive of alternate bone formation and resorption. Osteoid was found to cover the interdental crests and to line the socket wall in some areas, giving an impression of ankylosis. However, close examination revealed a narrow strip of periodontal membrane separating the root cementum and the alveolar osteoid (Figs. 5, 6).

The principal fibers of the periodontal membrane presented a deep basophilic staining in the vicinity of the cementum of the root. In some areas these basophilic fibers

FIG. 6.—Same section as in Fig. 5 but of control animal

appeared fused. Sharpey's fibers, also, took on a dark stain, suggestive of their calcification. In addition, calcified irregular bodies were seen in some areas of the periodontal membrane.

The incisor periodontium revealed central alveolar bone resorption and osteoid deposition both endosteally and on the socket wall (Fig. 7). Again a pagetoid pattern, indicative of repeated waves of remodeling, was observed. On the other hand, calcifications were not found in the incisor periodontium, nor were significant changes seen in the dentin or pulp tissue, of the incisors.

DISCUSSION

In the hamsters given excess vitamin D there was a continual drop in body weight, anorexia, hypercalcemia, pathologic calcification in the kidney, and both erosion and reduced calcification of the femur. These reactions are typical of the hypervitaminosis D syndrome as it is seen in other animals and establishes that the desired condition was obtained in the hamsters.

The level of plasma calcium, 12.2–12.5 mg per cent for the control animals, corresponds with values obtained by other investigators,[11] and the value of 15.9 mg per cent for the experimental animals is not unlike that found in hypervitaminosis D in other animals.

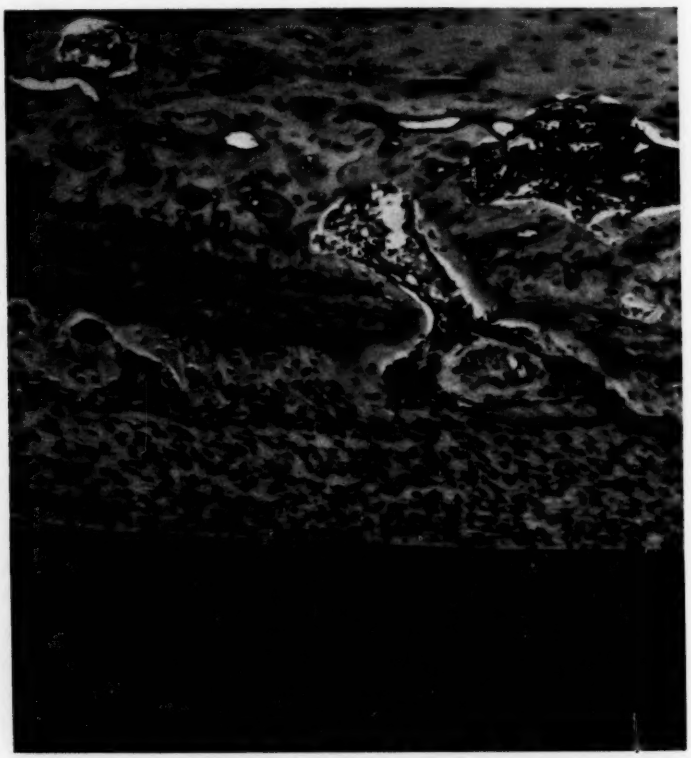

FIG. 7.—Labiolingual decalcified section of the right mandibular incisor and lingual periodontium of hamster given excess vitamin D. Note the pagetoid remodeling of bone. (Approximate mag. ×100.)

It was desired that the amount of vitamin D administered to the hamsters should provide a well-developed syndrome in 8 weeks. The amount chosen was based on work which showed that the rabbit, which is relatively sensitive to vitamin D overdosage, survived about 6 weeks on a regimen of 44,000 I.U./kg/ biweekly.[12] This dose was administered to the hamsters. The results indicate that the hamster is somewhat more resistant than the rabbit, but that the vitamin D level was appropriate to this type of study.

In general, the picture of hypervitaminosis D in the oral cavity of the hamster has the following features: calcified deposits in the periodontal membrane, cemental spurs, calcification of the principal periodontal fibers, and resorption of alveolar bone. These effects are qualitatively similar to those seen in other animals exposed to excessive amounts of vitamin D. Now this systemic effect may be induced with ease and assurance in large numbers of hamsters with measurable amounts of periodontal disease. Thus, with established scoring procedures, one can determine whether the systemic factor plus the local factor influences periodontal disease scores.[9]

SUMMARY

The initiation and development of periodontal disease may be dependent on systemic factors, such as the calcium load of the plasma and soft tissue, and conditions due to an excess or deficiency of vitamins and hormones. It was demonstrated in this study that excessive amounts of vitamin D markedly modified the periodontal tissues in young adult hamsters. Further studies of these effects in hamsters with periodontal disease are warranted.

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Testing of Antioxidants against Experimental Caries in Rats

H. V. JORDAN, A. E. BOWLER, and N. D. BERGER

National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland

Demonstration of the caries-inhibiting potential of sodium metabisulfite[1] has been followed by subsequent investigations into certain other aspects of the chemical activities of this compound in an effort to clarify its mode of action. For example, two other carbonyl-binding compounds—dimedone and aminooxyacetic acid hemihydrochloride—were also found to inhibit rat caries.[2] The well-known antioxidant properties of bisulfite[3] have prompted the testing of a number of other common antioxidants, including some used commercially in foods, for their effect on experimental caries produced by a coarse corn-particle diet. Of additional interest in this respect are certain reports[4, 5] concerned with the effect of oxidizing agents on experimental caries and on acid production in saliva. However, the exact role of these compounds is not clear, since varying results have been reported. For example, Hein and Wisotsky[4] found that low levels of potassium permanganate had no effect, while Shapiro, Brat, and Ershoff[5] reported that hydrogen peroxide and other oxidizing agents, including zinc peroxide and sodium perborate, initiated caries in rats on a normally non-cariogenic diet.

EXPERIMENTAL METHODS

Animal experiments.—The procedure used in the present work for testing various compounds for their effect on sulcal caries has been previously described in detail.[1, 2] Each experimental group was composed of 24 weanling, female, Sprague-Dawley rats which were randomly distributed by threes in screen-bottom cages. The cariogenic diet employed was the coarse corn-particle diet of Stephan, Fitzgerald, McClure, Harris, and Jordan.[6] After an experimental period of 90 days the animals were sacrificed, and the molar dentition was scored for sulcal caries, using the method of Cox, Dodds, Dixon, and Matuschak.[7]

The first study was composed of three experimental groups and a control group. Groups 1 and 2 received 0.5 per cent ascorbic acid and isoascorbic acid, respectively, in the diet. The third group received the synergistic antioxidant combination[8] of α-tocopherol and ascorbic acid at 0.05 and 0.1 per cent, respectively. The α-tocopherol was first dissolved in ether and then blended into the diet. Ether was allowed to evaporate by spreading the diet out in shallow pans placed in the chemical hood.

A second animal experiment was composed of 6 experimental groups and a control

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group. Group 1 received butylated hydroxytoluene (BHT)* at 0.5 per cent, and group 2 received 0.5 per cent of BHT and citric acid (50-50). The citric acid acts as a metal chelator to enhance the activity of the antioxidant. The third group received 0.25 per cent citric acid as a control for group 2. The fourth and fifth groups received, respectively, *n*-propyl gallate and ethyl hydrocaffeate† each at 0.5 per cent. Group 6 received 0.4 per cent antioxidant salt‡ at the expense of the NaCl normally in the diet. Ethyl hydrocaffeate, a thick liquid, was first dissolved in propylene glycol and then blended into the diet. All other supplements were mixed into the diet as dry powders.

The third animal experiment was also composed of 6 experimental groups and a control group. The following compounds were all tested individually at 0.5 per cent in the diet: α-tocopherol acetate, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EMQ), rutin, hesperidin, butylated hydroxyanisole (BHA),§ and nordihydroguaiaretic acid (NDGA). Alpha tocopherol and EMQ were first dissolved in ether before blending into the diet as previously described. BHA was obtained in the form of 10-gm. pellets, which were ground to a fine powder before use.

Bacteriological experiments.—The antioxidants were also screened for inhibition of the growth, in vitro, of a variety of test bacteria representing types encountered in the oral cavity. The solubility in water of many of the antioxidants was quite low, and they could be brought into solution or stable suspension for testing only under special conditions. The limits of solubility generally determined the highest concentrations tested. Stock solutions of the compounds were prepared as follows: *n*-propyl gallate, 2 per cent in distilled water with the pH adjusted to 8.3; ethyl hydrocaffeate, 2 per cent solution in 10 per cent propylene glycol; antioxidant salt, 4 per cent in distilled water with 10 drops of 10 per cent HCl added; isoascorbic acid, 2 per cent in distilled water; α-tocopherol acetate, 0.5 gm. mixed with 5 gm. Tween 80 added dropwise with mixing up to a total of 100 ml. in warmed distilled water, 6 drops of 10 per cent HCl added to clarify the mixture; butylated hydroxytoluene, 0.5 gm. dissolved in 1 ml. 95 per cent ethyl alcohol, mixed with 6 gm. Tween 80 and made up to a total of 100 ml. with warmed distilled water, 6 drops of 10 per cent HCl added to clarify the mixture; BHA, prepared in the same way as BHT; EMQ, 1 gm. mixed with 0.5 gm. Tween 80 and made up to 200 ml. with distilled water; 4N HCl added drop-wise to clarify the mixture; NDGA, 0.5 gm. in 200 ml. distilled water with 10 drops of 10 per cent NaOH; rutin, 1.0 per cent in warmed distilled water with 10 per cent NaOH added dropwise to effect solution. All stock solutions were sterilized by filtration|| and added aseptically to the previously autoclaved medium. The details of testing, such as serial dilution of the antioxidants, media used, inoculum size, etc., are exactly the same as described previously.[1, 2] Appropriate controls were included for the various solubilizing agents employed. In tubes where no growth occurred the pH was determined, to make sure that it did not vary significantly from that of the basal medium.

* 2,6-Di-tert butyl *p*-cresol.

† Ethyl ester of 3,4-dihydroxy phenylpropionic acid.

‡ The percentage composition of antioxidant salt is butylated hydroxyanisole, 0.128; propyl gallate, 0.047; citric acid, 0.028; propylene glycol, 0.047; tricalcium phosphate, 1.5; sodium chloride to make 100 per cent.

§ 2-Tert butyl-4-hydroxyanisole.

|| Corning U-F sintered-glass filters were used for this purpose.

RESULTS

The data for the three animal experiments are presented in Table 1. Only in the cases of propyl gallate in experiment 2 and EMQ in experiment 3 was there an appreciable reduction in the caries scores. Approximately a 50 per cent reduction was observed in each case. The mean caries scores in some of the groups were higher than the control scores. This was seen in the ascorbic acid, isoascorbic acid, BHT + citric acid, citric aid, rutin, and hesperidin groups. The caries reductions by propyl gallate and EMQ and the caries increase in the hesperidin group were found to be statistically significant at the 0.1 level. BHT and EMQ were apparently toxic at the levels employed. Because of unsatisfactory weight gains, the initial concentrations were reduced to half

TABLE 1

EFFECT OF ANTIOXIDANTS ON GROWTH AND SULCAL CARIES IN RATS WHEN TESTED ON DIET 585 FOR 90 DAYS

Expt. No.	Group	Compound	Conc. in Diet (Gm/kilo)	Av. Final Wt. (Gm.)	Av. Daily Food Intake (Gm.)	Survivor Ratio	Av. Carious Teeth	Av. Caries Surface	Av. Caries Score	S.E. (±)
I.....	1	Ascorbic acid	5	196.0	10.3	21/24	4.1	10.2	21.6	2.57
	2	Isoascorbic acid	5	193.0	10.0	21/24	3.9	9.5	20.4	2.84
	3	α-Tocopherol + ascorbic acid	0.5 and 1.0	204.2	9.9	24/24	3.6	7.7	15.1	2.04
	4	Control	.....	203.7	9.0	24/24	3.8	7.5	13.8	1.95
II....	1	Butylated hydroxytoluene (BHT)	5*	169.1	7.9	21/24	4.0	9.1	17.6	2.82
	2	BHT + citric acid (50/50)	5*	185.6	8.7	20/24	4.0	10.0	21.1	2.44
	3	Citric acid	2.5*	197.5	9.8	21/24	4.4	12.5	28.6	3.77
	4	*n*-Propyl gallate	5	178.7	9.5	23/24	3.0	5.2	9.7	1.76
	5	Ethyl hydrocaffeate	5†	196.9	9.6	21/24	4.5	8.3	15.6	1.96
	6	Antioxidant salt	0.4	196.1	9.5	22/24	3.6	9.3	17.0	2.65
	7	Control	.....	201.5	9.9	22/24	3.5	9.1	18.5	2.55
III...	1	α-Tocopherol	5	203.6	9.2	24/24	4.2	8.2	16.1	2.07
	2	1,2-Dihydro-6-ethoxy-2,2,4-trimethyl quinoline (EMQ)	5	169.7	9.4	19/24	2.9	4.7	7.2	1.91
	3	Rutin	5	194.1	8.1	22/24	5.1	11.5	23.8	2.71
	4	Hesperidin	5	201.6	9.0	22/24	4.4	10.3	22.5	2.61
	5	Butylated hydroxanisole (BHA)	5	193.2	8.4	24/24	3.9	8.5	19.5	4.38
	6	Nordihydroguaiaretic acid (NDGA)	5	168.0	11.3	24/24	3.8	7.9	17.2	2.71
	7	Control	.....	206.6	9.3	23/24	4.1	8.0	14.8	1.62

* Reduced to half after 1 week.
† Reduced to half after 3 weeks.

after the first week for the groups receiving BHT alone and in combination with citric acid and after the third week for the EMQ group. The average final weight for animals in these groups was still considerably lower than the control animals. Feeding propyl gallate and NDGA at the levels employed also resulted in reduced weight gains. No excessive mortality was noted in any of the groups, with the possible exception of the EMQ group. The toxicity of this antioxidant has been more completely investigated by Wilson and DeEds.[9]

Characteristic stains were observed on the molar teeth of the rats fed propyl gallate. The exposed dentin on the occlusal surfaces of the cusps exhibited a brownish discoloration. In addition, the depths of the sulci, cracks in the enamel, and the gingival line on the molars in many cases were stained a deep black. This may be a consequence of the ability of propyl gallate to form dark precipitates with certain metals in the diet. Essen-

tially the same picture, although to a much lesser degree, was seen in the group fed ethyl hydrocaffeate. Teeth from both groups were sectioned, and the staining appeared to be confined to the surface layers of the dentin. The dried skulls and teeth of animals from the hesperidin and rutin groups were examined under ultraviolet light. The characteristic induced bone fluorescence reported to result from quercetin ingestion[10] was not observed.

Only 4 of the 13 antioxidants and antioxidant preparations showed any appreciable inhibition of the test bacteria at the concentrations tested. Inhibitory levels of propyl

TABLE 2

INHIBITION OF SELECTED BACTERIA BY FOUR ANTIOXIDANTS*

Micro-organism	Strain No.	Propyl Gallate	E.M.Q.	Ethyl Hydrocaffeate	Nordihydro-guaiaretic Acid
Lactobacillus arabinosus	17–5	0.266	>0.10†	>0.40†	0.050
L. plantarum	8014	0.266	>0.10	>0.40	0.050
L. casei	7469	0.029	>0.10	>0.40	0.050
L. fermenti	36	0.088	>0.10	0.40	0.050
L. fermenti (rat)	NR77	0.266	>0.10	>0.40	0.050
L. acidophilus (rat)	42T	0.088	>0.10	>0.40	0.050
Candida albicans	358	0.266	0.033	0.40	0.05
C. albicans	394	0.266	0.033	0.40	0.05
C. pseudotropicalis	360	0.266	0.033	0.40	0.017
C. krusei	359	0.266	0.10	0.40	0.05
C. krusei	379	0.266	0.033	0.40	0.05
Neisseria catarrhalis	N6	0.0032	0.011	0.014	0.006
N. flava	N8	0.0032	0.10	0.014	0.017
Streptococcus salivarius	9756	0.266	>0.10	>0.40	0.017
S. salivarius	3	0.266	>0.10	>0.40	0.017
S. faecalis	R	0.266	>0.10	>0.40	0.017
S. milis	2	0.266	>0.10	0.40	0.017
Streptococcus (rat)	FA-1	0.088	>0.10	>0.40	0.017
Streptococcus (rat)	3R-114	0.088	>0.10	>0.40	0.017
Staphylococcus aureus	13	0.088	0.033	0.40	0.017
Staphylococcus aureus	33	0.088	0.033	0.40	0.017
Gaffkya tetragena	10875	0.029	>0.10	0.13	0.006
G. tetragena	J113	0.029	>0.10	0.13	0.017
Micrococcus	19	0.088	>0.10	0.13	0.017
Micrococcus	6	0.088	>0.10	0.043	0.017
Leptotrichia	Sp.	0.029	0.0037	0.130	>0.05†
Leptotrichia	Sp.	0.029	0.011	0.130	>0.05

* Inhibitory levels are expressed as per cent antioxidant in the first tube which inhibited growth.
† Highest concentration tested.

gallate, EMQ, ethyl hydrocaffeate, and NDGA are shown in Table 2. It can be seen that EMQ and ethyl hydrocaffeate were only partially inhibitory to certain of the bacteria at the highest concentrations tested. With NDGA there was a difference in the relative sensitivities of streptococci and lactobacilli, the former being inhibited at a lower level than the latter. This antioxidant was reported to have no effect on bacterial counts in prepackaged meat.[11] The two staphylococci were more sensitive to EMQ and less sensitive to ethyl hydrocaffeate than were the gaffkyae and other micrococci. The neisseriae were generally more sensitive to these antioxidants than were the other bacteria. Isoascorbic acid at 0.266 per cent, α-tocopherol at 0.1 per cent, BHT at 0.1 per cent, and antioxidant salt at 0.4 per cent did not inhibit growth. BHA was generally ineffective at 0.2 per cent except for strains of leptotrichiae and neisseriae, which were inhibited at this concen-

tration. Rutin was not inhibitory to any of the bacteria at concentrations up to the limit of its solubility of around 0.2 per cent. Naghski, Copley, and Couch[12] had previously reported the lack of antibacterial activity for rutin against various bacteria. Gustafsson[13] has reported the failure of glycosides of various flavonols to inhibit acid production from glucose in saliva while the aglycones were active. Naringenin was later shown to inhibit experimental caries in hamsters.[14]

DISCUSSION

The widespread use of antioxidants,[15] especially in foods, makes any knowledge of their physiologic effects of particular interest. In the present work these compounds were purposely tested at considerably higher concentrations than are normally used to stabilize foods. Even at these levels it seems apparent that antioxidants as a class do not possess any marked degree of caries-inhibiting properties under the conditions used for testing. Thus it would appear that the antioxidant properties of metabisulfite are not the primary cause of its inhibitory effect on experimental caries in rats. It is probably significant that the two antioxidants which showed some caries inhibition were also two of the four compounds which could be demonstrated to inhibit the growth of selected test bacteria. Thus it may be that any interference with caries development by these two agents was simply an antibacterial effect rather than any direct antioxidant effect in the oral cavity. However, the low solubilities of the antioxidants in general, except under the special conditions noted in the in vitro tests, make it difficult to interpret the relationship between bacterial inhibition in vitro and caries inhibition in vivo.

The work of Shapiro *et al.*[5] in which oxidizing agents were reported to induce dental caries on a non-cariogenic diet is difficult to evaluate in relation to the present experiments. These workers described a destruction of the exposed dentin at the tip of the molar cusps, proceeding inward until the pulp was involved. In the present work, caries was initiated in the molar sulci, penetrating the enamel before involving the dentin, and hence the two processes may not be comparable.

SUMMARY

A number of antioxidants were tested for their effect on sulcal caries in rats. Propyl gallate and 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline inhibited caries by approximately 50 per cent when incorporated in the diet at 0.5 per cent level. The other antioxidants, including α-tocopherol, ascorbic acid, isoascorbic acid, butylated hydroxyanisole, ethyl hydrocaffeate, rutin, hesperidin, butylated hydroxytoluene, and nordihydroguaiaretic acid, did not inhibit caries. In fact, the caries scores of some groups were slightly increased. In the case of hesperidin, the increase was statistically significant. These compounds were generally not inhibitory to the growth of selected test bacteria under the conditions used for testing. Exceptions were propyl gallate, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline, ethyl hydrocaffeate, and nordihydroguaiaretic acid.

Samples of antioxidants were obtained through the generosity of the following companies: 1,2-dihydro-6-ethoxy-2,2,4-trimethyl quinoline from the Monsanto Chemical Co., St. Louis, Mo.; isoascorbic acid from Sumner Chemical Co., New York, N.Y.; antioxidant salt from Diamond Crystal Salt Co., St. Clair, Mich.; butylated hydroxytoluene from McKesson and Robbins, Inc., New York, N.Y., and Hercules Powder Co., Wilmington, Del. Other antioxidants were purchased from commercial sources. Teeth were sectioned through the courtesy of Dr. Rachel H. Larson. The technical assistance of Mr. William L. Poole is acknowledged.

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The Role of Electrical Potential Differences in the Etiology of Chronic Diseases of the Oral Mucosa

JÁNOS INOVAY AND JOLÁN BÁNÓCZY
Clinic of Oral Surgery, Medical University of Budapest, Hungary

For a long period it has been well known that electrical potential differences may develop between various metal fillings and metal restorations. Patients' complaints can be divided into two groups: (1) subjective complaints immediately following the placement of the metal filling, such as metallic taste or sensation, and (2) subjective and objective complaints, which develop after a certain time, such as chronic inflammation of the mucous membrane and those of neuralgic character.

Investigators have been interested in this problem for a considerable time. Chase[1] in 1879 attributed the destruction of the teeth to various metals. Lain[2] and, after him, others[3–5] measured this electro-activity. Regarding the origin and characteristics of the current, opinions vary. Most investigators[2, 6–10] explain it in terms of the formation of a galvanic cell, but recently much importance has been given to bioelectric powers.[11, 12] In Hungary, Tarnai and Záray,[13, 14] interested in bioelectric influence, measured in vitro the potential difference of certain metals relative to the calomel electrode in saliva.

Several authors have attributed oral damage to electric potential differences between various metals. Ullmann[16, 17] in 1932 reported a survey of 580 cases of leukoplakia in which cancer developed in three instances. Other studies[4, 5, 15] showed that the next most frequent illnesses were glossitis, cheilitis, perlèche, and neuralgic complaints.

Because the simultaneous use of dissimilar metals (precious metals, stainless-steel alloys, amalgam) is common in modern dentistry, their practical importance in the etiology of oral diseases is great. The aim of this investigation was to ascertain whether a certain connection could be established between the incidence of chronic inflammation of the oral mucosa, its subsequent healing, and the grade of electrical potential differences.

EXPERIMENTAL METHODS

In all cases where primary and secondary conductors contact (metals and electrolytes), galvanic cells are formed, and an electric current is generated by the precipitation of metal ions or their contact with solution in an ionized state. Oral electric sources also act as galvanic cells in the majority of cases. An electric source is characterized by its electromotive force (E.M.F.), which is the potential difference between the poles of the unladen electric sources, and its terminal voltage, which is the tension between the poles of the laden electric source. In our experiments we measured the

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terminal voltage, since this measurement gives certain information about the capacity of the electric source. During the generation of electrochemical and electrophysiological phenomena, working capacity—i.e., the output of the current—is more important than its potency. Certain other oral bioelectric potentials three times as small as the voltage generated by metals are considered to be insignificant. Potentials may arise between identical metals if the surrounding ion concentration is different or if a difference in the pH exists. In this paper we shall talk of concentration elements. Thermoelectric efficiency, electro-osmosis, and membrane potentials were neglected because of their order of magnitude.

Measurements were made with the highly sensitive (10 K Ω/V) Deprèz instrument. Copper and later platinum electrodes were used. The terminal voltage was measured in every instance between each metal part, and the potential differences between the

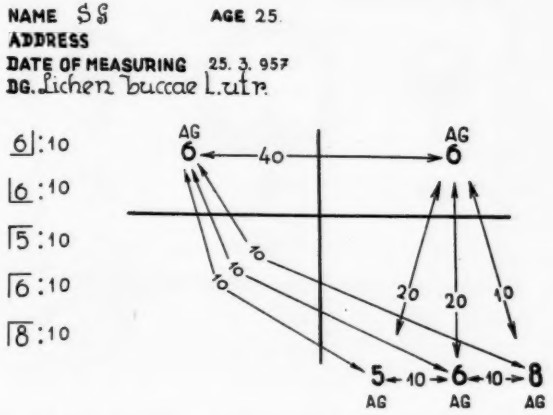

FIG. 1.—Electrogram showing dental status of investigated person. Potential differences found between metal restorations and fillings are also recorded. The electrogram contains the most important data of the patient.

metal parts and the tongue were also determined. The following equation gives the number of measurements in case of n number of metals:

$$M = \frac{n(n-1)}{2} + n.$$

Measurements for one individual are shown in Figure 1, in which the dental status of the patient is also given. To avoid electrode polarization during measurements, a high-power inertia instrument, such as the ballistic galvanometer apparatus, was used. Measurements were repeated after 1 hour, when repolarization could be assumed. Measurements of electric power were obtained by means of a self-constructed, battery-fed, electronic tube voltmeter (Fig. 2).

Measurements were made on 69 subjects, in which the following clinical diagnoses could be established: leukoplakia, 22 cases; lichen, 16; glossitis, 21; cheilitis, 2; neuralgia, 2; gingivitis, 2; and metallic taste, 4.

In each case a detailed history was taken and general examinations made. To complete clinical examinations, the necessary laboratory tests (blood count, BSR, test

meals, Wassermann reaction, X-ray, and focal examinations) were also performed. The majority of the cases had been under treatment for a considerable time, but when therapy failed to bring the desired result, the potential differences between the various metals in the oral cavity were measured.

The dental status of the subjects was also determined. In cases where differences in potential were found, a schedule was drawn for the sequence of the replacements, exchanging, first, the restoration which showed the highest potential difference. Control measurements were made after a period of 2 years, at which time the condition of the oral mucosa was again recorded.

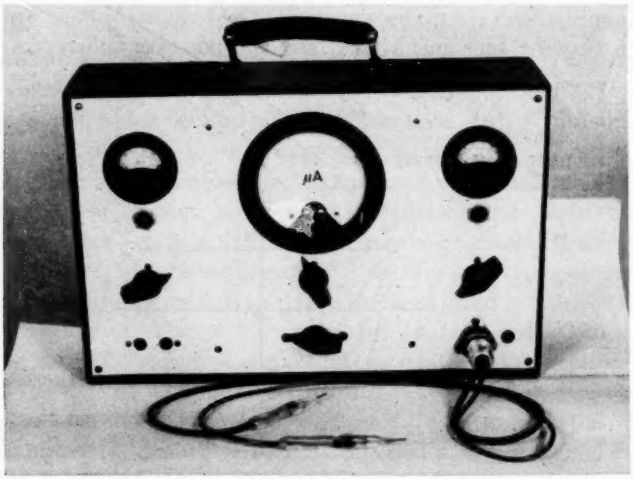

FIG. 2.—Self-constructed battery-fed electronic-tube voltmeter for the estimation of E.M.F. (electromotive forces). The instrument is supplied with input of high impedence. Its sensitivity for total deflection is 200 mV. It is furnished with platinum electrodes.

RESULTS

Repeated measurements were made in 109 instances on 69 subjects. The total number of measurements was 1,601; of these, 1,199 were made between the restorations in the oral cavity and 402 between the restorations and the tongue. The number of measurements made for each subject varied from 1 to 42, according to the number of metal fillings; on the average, 14 measurements per patient were made.

Table 1 shows the maximal and average values in mV noted between the various metals. Highest values were found between stainless-steel and amalgam restorations. In 82 instances a potential difference above 50 mV was measured. These values were regarded as pathologic. Table 1 also indicates that, contrary to previously published data and the general view, relatively high potential differences were found between identical metals. Further experiments and measurements are necessary to explain this phenomenon.

When measuring the difference between the tongue and some metal part, in 22 cases out of 402 a value surpassing 50 mV was found. The highest value was 140 mV.

Control examinations were made in 40 cases. Among these, 16 had, following advice,

had their metal restorations replaced. Of these 16 cases, potential differences diminished or disappeared in 15 cases, and 1 case remained unchanged, presumably because of a new filling in the oral cavity.

Upon control examination, the status of the oral mucosa was compared with the previously obtained records. Cases in which a change in the dental status due to metals was found were separately registered. Changes in oral damage are recorded in Table 2. In those cases in which the metals with galvanic action were removed, the number of healed or improved cases was higher than in cases where such exchanges were not made.

TABLE 1

POTENTIAL DIFFERENCES ESTIMATED IN ORAL CAVITY BETWEEN VARIOUS METALS
(Number of Measurements, Maximal and Average Value)

	Stainless Steel–Stainless Steel	Amalgam-Amalgam	Stainless Steel–Amalgam	Gold-Amalgam	Gold–Stainless Steel	Gold-Gold	Total
No. of measurements	301	212	238	151	176	199	1,199
Maximal value (mV)	120	120	300	130	80	40	.....
Average value (mV)	21	17	25	20	19	12	.....
No. of measurements above 50 mV	18	10	36	14	4	.....	82

TABLE 2

EFFECT OF EXCHANGES OF METAL RESTORATIONS UPON ORAL DAMAGE

ORAL DAMAGE	DENTAL STATUS UPON CONTROL EXAMINATION	
	Changed	Unchanged
Unchanged	3	13
Improved or healed	13	11
Total	16	24

Case Reports.—W.R., a woman aged sixty-six, came to the clinic in 1956. She complained of a painful abscess on the right bucca. Prior to this, lichen ruber planus was diagnosed at a dermatological department, on the basis of a rash on her hands and feet. Papular lesions characteristic of lichen, found bilaterally, supported the diagnosis, and the erosion on the right bucca seemed to be of the same origin. It was about 2 cm. in diameter, bled upon contact, and was very painful. It disturbed the patient when eating, which resulted in loss of weight. After the usual arsenic treatment, administration of A, B, and C vitamins, and epithelizing salves, the erosion showed only a slight improvement but broke out afresh upon discontinuance of the treatment. After an unsuccessful treatment of about 18 months, measurements of electrical potential differences in the oral cavity were made. A potential difference of 300 mV was found between an amalgam filling in the lower-right second bicuspid and a stainless steel bridge connecting the lower-left first bicuspid and second molar; 120 mV between an amalgam filling in the lower-right second bicuspid and a gold crown on the upper-right cuspid; 60–60 mV between

the amalgam fillings of the lower right first bicuspid and upper-left central incisor. Upon the extraction of a seriously damaged lower-right second bicuspid, epithelization of the erosion was noted. Upon removal of the gold crown from the upper-right cuspid, an amalgam filling was found which was replaced by cement and the tooth covered with an acrylat crown. Control measurement showed a potential difference of only 20 mV. The erosion healed in 2 weeks and has not recurred during the last 3 years. The patient is free of complaints.

U.A., a man aged fifty-five was referred to the authors by his dentist with a leukoplakia on the left bucca. The patient neither smoked nor drank, and his Wassermann reaction was negative. Upon examination, a hyperkeratosis of 2 cm. in diameter was found on the left bucca, in the corner of the mouth. He had four stainless-steel bridges and numerous amalgam fillings. Measurements revealed 100-, 70-, 70-, and 90-mV potential differences between the amalgam filling in the lower-left first bicuspid and the stainless-steel bridges. The amalgam filling in the lower-left first biscuspid was replaced by cement. Upon control examination after 2 weeks, leukoplakia was already in total regression. Control measurements showed maximal values of 20–30 mV.

DISCUSSION

The 1,601 measurements led to the conclusion that, in numerous cases, high potential differences can occur between similar metals. This may be due to the following causes: (1) Metals or, to be more exact, alloys which are considered homogeneous are of different structure. This explanation might apply to amalgam fillings, where the mode of preparation, i.e., the Hg-Ag-Sn composition, may vary considerably. (2) Potential differences can be encountered even within the same bridge when, because of unsatisfactory heat treatment, the crystal structure is not homogeneous.[18]

On the basis of the present experiments it can be stated that, in the case of bridges soldered from different metals, potential differences cannot be found, since such a bridge is a short-circuit electrical source, where a flow exists, but because of low internal resistance the terminal voltage is zero. If, however, the soldering cracks, the short circuit ceases, and a potential difference between the two metal parts of the bridge can be discerned.

The variability in the surface finishing and polishing of identical metals should also be taken into consideration, since that may also influence the different electrochemical behavior of metals.

Fillings under crowns of which the dentist is ignorant may considerably influence the electrical behavior of the crowns. Of primary significance are cases in which crown and filling are not in galvanic contact and the cement acts as secondary conductor. In such cases the crown functions as a separate galvanic cell.

Differences in the water content or pH of the cement may explain the potential differences experienced between gold inlays—the two gold inlays act as a concentration cell. The resulting potential difference is proportional to the natural logarithm of the ion concentration surrounding the two metals. This likewise refers to amalgam fillings underlined with cement or, in cases of identical metal crowns, on intact teeth.

During the measurement of current rates it was noted that after fixation of the electrodes—following a maximal deflection—the instrument gradually returned to its resting position. Because of the polarization of metal parts acting as small galvanic cells, the polarizing tension affecting the original E.M.F. diminishes the measurable potential. After a cut, metals repolarize, and the original potential difference is again measurable in about an hour. In the oral cavity this polarization of metal parts may

arise independently of measurement, when, for instance, in a case of occlusion two metal fillings are short-circuited by an antagonist bridge, which causes permanent intense electronic stream. Since this polarization takes place on the surface of metals, it changes their electrochemical behavior, and therefore, even in case of identical metals or alloys, a measurable potential difference develops between them.

On the basis of the present experiments, a parallel cannot be drawn between the severity of registered chronic oral damage and the magnitude of potential difference. The subject's individual endurance and constitutional reaction play an important role. A decline in the organism's defensive reaction may lead to the manifestation of various symptoms. The time factor is to be regarded as primarily important, since the unfolding of the complaints takes considerable time. Sometimes years pass until a definite clinical entity develops as a result of damage caused by potential difference between replacements. The same applies to changes in conditions following the removal of metal replacements. No essential proof was found for the solubility of metals. Metal poisonings, gastroenteritis, or emesis caused mainly by copper dissolved from gold-substituting copper alloys, as described in previous references, were not encountered.

SUMMARY

Potential measurements in the oral cavity were performed in 109 instances on 69 patients who suffered chronic disease of the mucous membrane and did not respond to the usual treatment. Measurements were made with a self-constructed electronic tube voltmeter. 1,601 measurements were made between the various metals. Values above 50 mV were regarded as harmful.

In some cases, remarkable improvement was noted when harmfully high potentials were eliminated. The gravity of pathologic changes in the mucous membrane was not always in proportion to the magnitude of potential differences; the individual response of the organism was decisive.

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A Method for Study of Penetration of Enamel by Acid Buffers

R. S. MANLY, W. H. EMERSON, and K. K. KAPUR

Research Department, Tufts University School of Dental Medicine, Boston, Massachusetts

The effect of acidic buffers on tooth enamel has been studied previously by means of two general approaches. The window technique as developed by Brudevold[1] was used with slight modification by several others, including Ericsson,[2] Sullivan,[3] Crouse,[4] and Stralfors.[5] This approach follows decalcification by analysis of the buffer as the enamel dissolves. Decalcification can also be studied by observing changes in a property of the enamel. Enright, Friesell, and Trescher,[6] Besic,[7] and Coolidge, Besic, and Jacobs[8] have shown that treatment of enamel by sterile solutions is capable of producing histologic changes which closely resemble the pathologic changes of smooth-surface dental caries. Hals, Morch, and Sand[9] demonstrated that lactate buffers, pH 4.1–5.3, bring about subsurface birefringence changes in dental enamel in vitro. Each of these investigators performed his operations on some portions of tooth crowns, subjecting these to the action of a single immersing solution and either sectioning the pieces later for microphotography or analyzing the etch solution to determine changes in phosphate content.

There would be advantages in a method that would permit study of decalcification of thin tooth sections, provided that some means could be found to limit decalcification to a small area of the original external enamel surface. Such procedure would permit studies on different sections of the same tooth and serial observations by photomicrography on the same sections at different periods after the acid attack began. It would also eliminate the possibility of artifacts occurring during the sectioning. The use of several sections of the same teeth would afford controls that would permit efficient design of experiments. The goal of this investigation was to develop a procedure by which enamel sections could be imbedded in plastic with only the outer enamel surface exposed to the buffer and with the section mounted in a manner which would permit repeated photomicrography.

EXPERIMENTAL METHODS

Anterior teeth were obtained from the clinics of Tufts University School of Dental Medicine and Boston City Hospital. The teeth were cleaned of debris with a dull knife and placed immediately under moist conditions in a refrigerator. Sections of the crowns of the teeth were made at thicknesses of from 250 to 300μ with a lathe,* using a car-

This material was presented in part at the March Meeting of the I.A.D.R. in San Francisco, 1959.

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* Manufactured by George F. Motter and Sons, York, Pa.

borundum disk which rotated under a continuous stream of water. Sections were lapped individually on a horizontal lapping machine* to produce a thickness of either 125 or 200μ, a range that was optimum for observation under crossed polarized light with a first-order red plate, and the section oriented in the addition position. Control of thickness was obtained by the vernier caliper on the lapping machine and was checked later by a micrometer. The technique involved mounting the section with plastic between two glass slides, with some arrangement for exposure of a segment of the outer enamel surface. Several different resins were tried, but only one was found to be suitable.† Since the chief difficulties arose in exposing the external surface of the enamel, various mounting procedures were tried to determine which would provide uniform exposure of enamel, with least modification of its surface properties. The following procedures are presented in order of development. The rubber technique was found to be preferable and is employed at present.

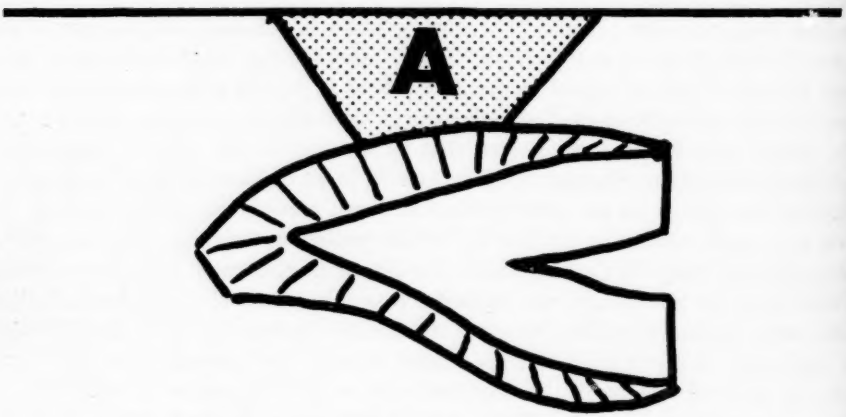

FIG. 1.—Shaded area illustrates position of wax mask for the wax technique

A. *Slice technique.*—Sections were mounted between glass slides with epoxy resin and with the labial or buccal surface of the section positioned at 1–2 mm. from the edge of the slides. After the resin had hardened, a notch was prepared by cutting away the plastic between the slides to expose the outer enamel surface. This was accomplished by means of a sandpaper disk, thinned on its outer edge, mounted on a contra-angle handpiece, and attached to a pantograph. This operation was performed with the aid of a binocular microscope. Remaining specks of plastic were removed with a sharp steel instrument.

B. *Wax technique.*—A section was mounted on the slide by means of a drop of epoxy resin, care being taken to prevent the resin from covering the external enamel surface. A small amount of wax (Fig. 1, area *A*) was then placed against the surface of enamel as a rectangular mask, extending to the edge of the slide. Special attention was given to make sure that no wax covered the top surface of the section. Another slide was then sealed to the top of the section by means of an epoxy resin. After the

* Manufactured by George F. Motter and Sons, York, Pa.

† Formex C2, obtained from Travaco Laboratory, East Boston, Mass.

resin had set, the slides were boiled, to flush out the wax and to create a slot for the buffer solution to enter. Sections were checked for the presence of plastic or wax covering the enamel by examining the slot between the slides through a binocular dissecting microscope, and any covering was removed.

C. *Rubber technique.*—Sections were prepared in a manner identical with that used for the wax technique except that rubber-base impression material was used instead of wax.* This could later be picked out with an explorer, and the section was checked on the dissecting microscope.

D. *Experimental plan.*—The first approach was concerned with the rate of penetra-

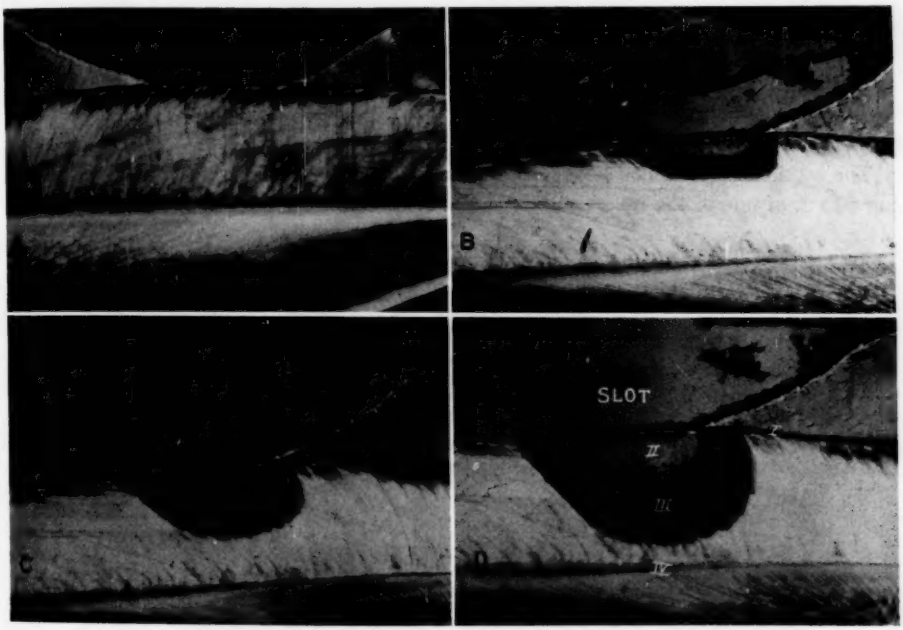

FIG. 2.—Typical example of cavitation and decalcification with 1 M lactate, pH 5. *A* = no treatment; *B* = treatment for 190 hours; *C* = treatment for 333 hours; *D* = treatment for 500 hours. The distinguishing features are the enamel surface (*I*), the gray area of cavitation (*II*), the black area of decalcification (*III*), and the dento-enamel junction (*IV*).

tion of enamel surface at different intervals after immersion of a section in buffer. Repeated observations and photomicrographs were made daily until decalcification had penetrated to the dento-enamel junction. Four variables for study were concerned with the composition of buffer used in the investigation. These were the pH, molarity, degree of saturation, and kind of acid employed. Two additional variables were concerned with the method of preparation of the section or the manipulation during tests. These were the degree of agitation and the depth of the slot through which the buffer reached the enamel surface. Surface alteration was found to be an important variable, which had a great effect on the rate of penetration of the buffer. Studies on these rates of penetration with different acids in the buffer will be presented at a later date when

* Sta-Tic, Stalite Incorporated, 4170 Park Aveue, New York 57, N.Y.

sufficient data with an acceptable technique are available. Nearly all the experiments were designed to study simultaneously three variables. Two of these variables were molarity and degree of saturation (all solutions were at pH 5), and a third variable was studied at two levels. In experimental design terminology this plan would be called a $2 \times 4 \times 4$ experiment. It requires 32 sections for one experiment. Usually no more than 6 sections could be obtained from the same tooth. The available replicates were distributed in a manner which would give the greatest precision for the variable that was studied at two levels.

E. *Preparation and use of solutions.*—Solutions were prepared from C.P. lactic acid, prepared to be 1 molar, and adjusted to pH 5 with sodium hydroxide. Four different molarities were prepared from this: 1.0, 0.5, 0.25, and 0.125. Each of these molarities was in turn prepared at four different degrees of saturation: 0, 25, 50, and 75 per cent, by saturating lactic acid with tricalcium phosphate for an hour at room temperature with stirring, and mixing the corresponding saturated and unsaturated solutions in the proper proportions.

The sections sandwiched between glass slides were immersed in coplin jars containing 60 ml. of one of the buffer solutions and set in an incubator at 37° C. Color trans-

FIG. 3.—Preparation of slot (*shaded area*) for slot-depth study

parencies* were taken for each mounted slide in the addition position under a polarizing microscope, using a first-order red plate and a 32-mm. objective. This initial transparency served as the control (Fig. 2, *A*). Additional transparencies were obtained daily during week days for a period of 1 month (Fig. 2, *B*, *C*, and *D*). The distance of penetration in millimeters and the degree of cavitation in millimeters was obtained by projecting the color transparencies at the standard magnification of 250 times and measuring the change with a millimeter scale.

RESULTS

A. *Influence of slot depth on penetration rate.*—This study was undertaken with an abbreviated experimental design involving only three molarities and three degrees of saturation. For these studies the tooth was mounted with one edge of the curved enamel surface flush with the edge of the slides, rather than recessed; then the exposure of the

* Kodachrome, Eastman Kodak Company, Rochester, N.Y.

slot was made along the curvature of the section to reach a depth of 3 mm. (Fig. 3). Readings on penetration of enamel were obtained at the edge flush with the slides and in the bottom of the slot, representing the minimum and maximum depths of slot.

Five solutions were studied—1 molar lactate buffer at pH 5, tested at 0 and 75 per cent saturation; 0.5 molar buffer at 50 per cent saturation; and 0.125 molar buffer at 0 and 75 per cent saturation.

Ten sections were employed for these studies, with five each being derived from two teeth. This number seemed to be ample for the investigations, since each section served as its own control. Each column in Figure 4 represents the mean of two readings, expressed as mm/1,000 hours and obtained from the penetration at 190 hours. The style

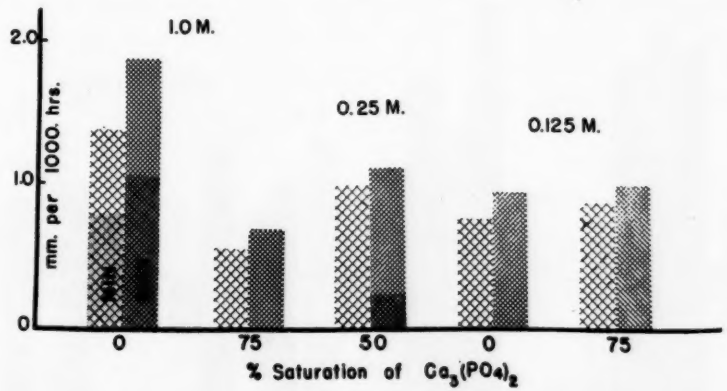

FIG. 4.—Influence of slot depth on rate of penetration and cavitation by lactate buffer. Penetration appears to be greater at the bottom of a slot.

FIG. 5.—Example of similar penetration rates at varying slot depths

of crosshatching shows whether the observations were made at a minimum slot depth (flush with edge) or maximum slot depth (3 mm. deep).

The first comparison was concerned with the influence of slot depth, the major variable under consideration. In all five instances the average penetration at maximum depth was greater than the penetration into enamel which was flush with the edge of the glass slides (Fig. 5). This finding is completely contrary to what might be expected on the basis of diffusion laws; yet the same observation was made concerning the degree of cavitation. With concentrated solutions and a low saturation, cavitation appeared first at the bottom of the slot and progressed more rapidly than it did near

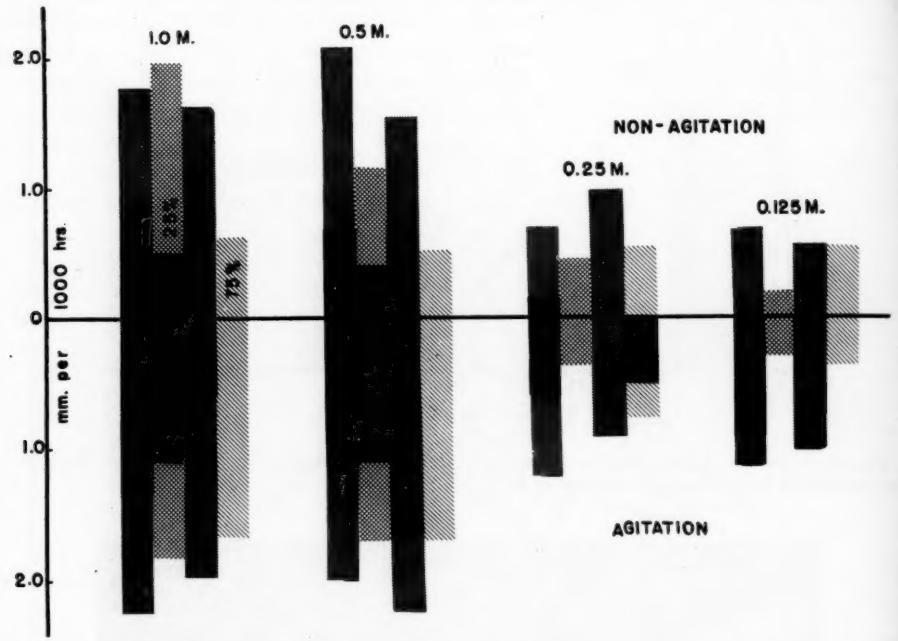

FIG. 6.—Influence of agitation on rate of penetration and cavitation induced by lactate buffer. Agitation appears to induce greater cavitation.

the surface. Some of the theoretical aspects of this finding will be discussed later, but it was obvious that this finding simplified experiments dealing with rate of penetration or cavitation, since they justified the technique of studying a section which was in contact with acid at the bottom of the slot.

Two inferences can be drawn from rate of penetration with the five solutions studied. With 1 molar buffer, a high degree of saturation produces a considerable decrease in rate of penetration. With low molarity of buffer this difference does not hold; the three-quarter-saturated buffer penetrates about as well as does a solution with no prior saturation. Probably buffers with low molarity are promptly saturated by contact with the enamel.

B. *Influence of agitation.*—It seemed possible that agitation of the solutions might increase the rate of penetration and cavitation of enamel. Agitation would remove,

from the enamel surface, buffer that had become partially saturated. The influence of agitation was studied by placing the coplin jars on a shaking platform in a water bath. The platform produced half-inch motion, 120 times per minute. In the first study with lactate buffer, 32 sections were employed in a 2 × 4 × 4 experiment. Half the sections were agitated, and the other half were not agitated. The results are presented in Figure 6. The penetration in millimeters per thousand hours was obtained from readings at 190 hours. Columns extending above the base lines show the penetration and cavitation for non-agitated solutions. Cavitation is represented by the darker area of hatching. The type of shaking shows the influence of increasing degrees of saturation. The groups of columns toward the right represent the action of more dilute buffer. In 11 cases out of 16, agitation produced a greater penetration, and, in all cases in which

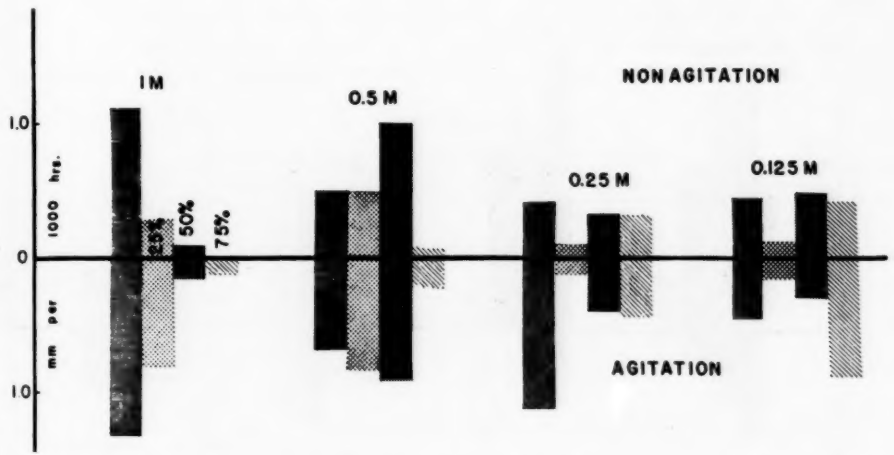

FIG. 7.—Influence of agitation on penetration by acetate buffer. Cavitation did not occur and agitation appeared to be unimportant.

cavitation occurred, it was greater for the agitated sections. The greatest effect of agitation seemed to occur with highly saturated solutions having a molarity above 0.5.

The same type of experiment was carried out for acetate buffer (Fig. 7). In this buffer there was no cavitation. There was no major advantage for the agitated specimens in their rate of penetration. Considerable variation is shown in the data of Figure 6, partly because the slice technique was employed and because the sections were selected at random.

C. *Wax method versus slice method.*—The comparison of the wax and the slice methods of exposing the original enamel surface is presented in Figure 8. This study involved two methods of preparation—the wax and slice methods—four molarities of lactate buffer, and four degrees of saturation. It would be classed as a 2 × 4 × 4 experiment. The pH employed was 5, and the data are based on observations made at 190 hours. Duplicates were averaged for each of the columns.

It is surprising to find that with 1.0 and 0.5 molar buffer, the slice method of preparing the section permitted a several fold greater penetration and cavitation than did the wax method. With $\frac{1}{4}$ and $\frac{1}{8}$ molar the difference seemed to disappear. This finding raises

the question as to whether there is unavoidable scratching of the enamel surface in the slice technique which removes a natural resistance of outer enamel to penetration or whether the wax technique leaves a thin but persistent coating that serves to increase the resistance of outer enamel. Possibly both these assumptions are true. The penetration of the wax-prepared sections was frequently delayed as long as 300 hours, but, once penetration had started, the slope of the penetration versus time curve (not shown) was almost identical with that of the sections prepared according to the slice technique. Hence the difference between the two techniques became insignificant, once penetration had developed through the outer layer and/or any coating on it. There still remained the question as to whether the persistence of the outer enamel surface

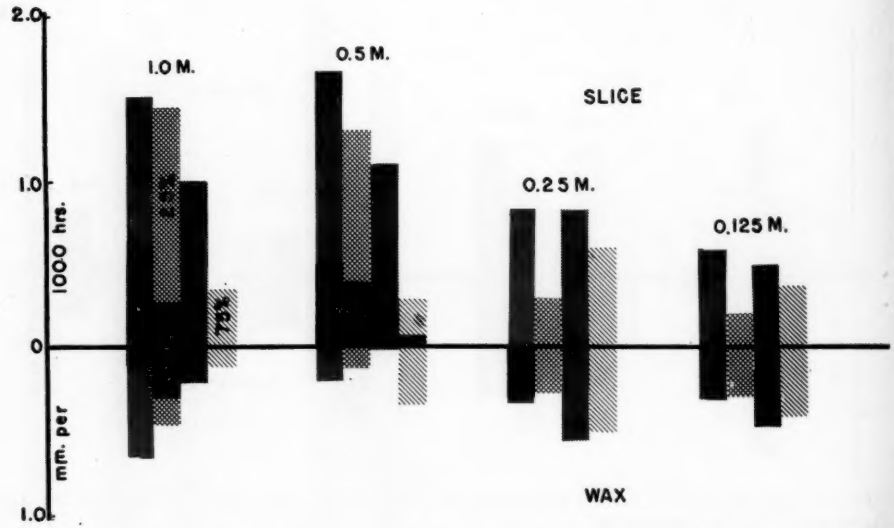

FIG. 8.—Comparison of wax and slice methods of preparing sections. The slice method generally leads to higher rates of penetration and cavitation.

was entirely due to some coating or whether it might have been caused in part by the lower solubility of the surface as described by Brudevold.[1]

The data in Figure 7 also permit a comparison to be made of the influence of molarity of buffer and degree of saturation. Because of questions regarding surface preparation, the data given in Figure 8 have to be interpreted qualitatively and with some reservations. The influence of buffer molarity appeared to be different for sections prepared by the wax and slice techniques. 1.0 and 0.5 molar solutions appeared to produce similar rates of penetration and higher rates than were produced by the 0.25 and 0.125 molar solutions. For sections prepared by the wax technique, molarity appeared to have very little influence, except that the 0.5 molar solution seemed to be less effective than any of the others. This was probably an artifact. The degree of saturation appeared to be important only with high molarities. More rapid penetration with increasing saturation could be seen with 1.0 and 0.5 molar solutions acting upon slice sections and for the 1.0 molar solutions acting upon wax preparations. Under all other

conditions the degree of saturation did not appear to be very important and in some cases actually showed a reversal of the expected results.

D. *Rubber versus wax techniques.*—The importance of this study rests on its ability to answer the question of whether the slice or wax techniques tend to cause artifacts in different directions. Six sections from each of two teeth were tested to get some indication of the influence of the pH of the buffer on the rate of decalcification. The sections from one tooth were prepared according to the rubber technique and distributed among 1 molar buffer solution having pH's of 4.0, 4.4, 4.8, 5.2, 5.6, and 6.0. Sections from the second tooth prepared by the wax method were distributed among the same buffers. The rate of penetration for the teeth prepared by the rubber technique is given in Table 1. The values ranged from 5.5 to 0.4 mm. per thousand hours, depending on the pH. Sections prepared by the wax technique showed no penetration at 333 hours. At this time the enamel surface was scraped gently with a steel instrument to remove any possible layer of wax, even though none was visible. The sections were reimmersed and checked again after 500 hours. All sections were immediately attacked

TABLE 1

RATE OF PENETRATION OF ENAMEL (MM/1,000 HOURS)

PREPARATION METHOD	pH					
	4.0	4.4	4.8	5.2	5.6	6.0
Rubber	3.9	5.5	2.2	0.7	0.4	1.7
Wax						
Before scraping*	0	0	0	0	0	0
After scraping*	6.0	2.0	4.0	4.6	2.1	1.4

* Enamel surface scraped gently with steel instrument after 333 hours of etch, values after scraping are calculated from this time.

and showed rates of penetration from 1.4 to 6.0 mm/1000 hours calculated from the initial point of 333 hours.

Apparently, the boiling or steaming required in the wax technique provided incomplete removal of wax, and the remaining wax impeded penetration of the buffer. Removal of wax by scraping the enamel surface resulted in a rate which was erroneously high because of disturbance of the enamel surface. These findings indicated that both the wax and the slice techniques were subject to artifacts and that the rubber technique was preferable.

DISCUSSION

There were several advantages in the use of tooth sections for a study of decalcification rates of dental enamel. Serial observations could be made on the rate of penetration with time, and the buffer or the enamel surface could be modified at any stage after decalcification was started and the effect of the modification on rate could be observed. Since several sections can be obtained from the same tooth and three or four experiments carried out on each section, it is possible to have as many as 30 areas of the same tooth involved in different experiments. Teeth can be modified in vivo by surface alterations, either chemical or physical, then sectioned and studied for resist-

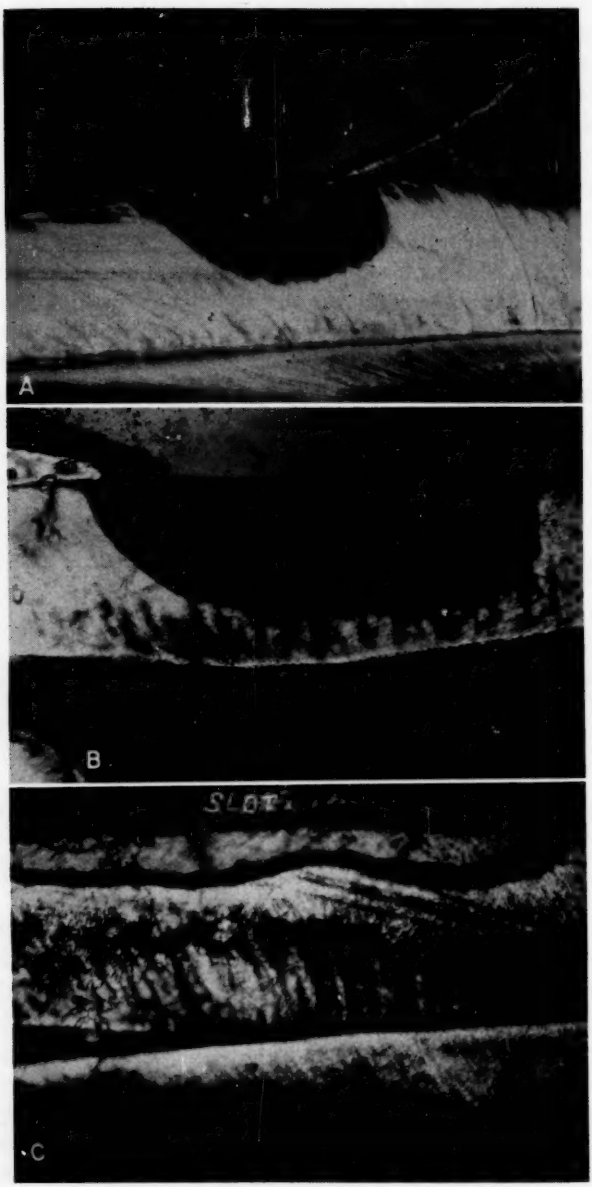

FIG. 9.—Examples of different behavior of lactate (*a*), acetate (*b*), and citrate (*c*) buffers. Acetate produces mainly decalcification, citrate mainly cavitation, and lactate is intermediate.

ance to a standard buffer. It seems likely that the use of plastic slides instead of glass slides will allow for Grenz-ray studies on the extent of decalcification.

Another advantage rests on the ease with which the area of modified enamel can be observed. This is disclosed by the contrast which exists in Figure 2, *A–D*. Figure 2, *A*, is the control picture taken before treatment. It can be projected and sketched on white paper, and the various stages of decalcification (Fig. 2, *B*, *C*, and *D*) can be projected on the same paper, and the distance of penetration can be estimated to the nearest micron by measuring with a ruler. These four slides are examples of the behavior of the section in pH 5 lactate buffer at different time intervals. Figure 9, *B*, represents an extreme decalcification which developed without cavitation and was obtained from a section immersed in pH 5 acetate buffer; the extreme of cavitation with relatively little additional penetration is observable in Figure 9, *C*, which was obtained when the decalcifying solution was citrate at pH 5.

Findings on agitation are probably of academic interest only. It is quite likely that little agitation occurs in solutions that are present in dental plaques. Stralfors[5] observed a four- to fivefold increase in solution of calcium and phosphate as the rate of shaking rose from 0 to 120 strokes per minute. The same speed of shaking was used in our studies, but the 3-mm. layer of liquid in the depth of slot was probably not so well stirred. The effect on cavitation was greater than on penetration, but our findings on relative cavitation with 0.25 molar buffer are in line with the effect which Stralfors observed.

The use of photomicrographs on tooth sections gives a permanent record of each experiment. The finding that cavitation and penetration are greater in the depth of the slot than in the absence of the slot is certainly puzzling from the standpoint of diffusion theory; yet it parallels clinical observations on the way dental caries progress in pits and fissures. This finding probably explains why decalcification can penetrate through to the dento-enamel junction without necessarily developing any cavitation. It now appears that decalcification and cavitation are independent and that cavitation tends to occur only when buffers are strong and the saturation is weak. Some preliminary data indicate that it also depends on the type of acid, in that it occurs much more frequently with lactic acid than with acetic acid.

SUMMARY

A procedure was developed for imbedding tooth sections between glass slides in a manner to leave 3–5 mm. of outer enamel surface exposed. These sections were studied for rate of penetration of pH 6 lactate buffers into the enamel, as recorded by photomicrography of the sections under polarized light. Epoxy resin was used to cement the section between the slide, and a rubber-base impression material was used to maintain an opening to the area under observation. A series of experiments was carried out with various molarities and degrees of saturations of lactate buffer and acetate buffer, to study certain variables associated with the imbedding technique.

Agitation was observed to be generally effective in increasing the rate of penetration of enamel. The influence of depth of slot on rate of penetration was studied, and it was found, contrary to expectation, that the rate was greatest at the bottom of a 3-mm. slot. The use of wax to prevent coverage of enamel by epoxy resin was found to be

unsuitable because the wax frequently left an impermeable coating on the enamel surface.

Strong solutions of buffer (1 molar) generally caused more cavitation and penetration than did weaker solutions. High degree of saturation caused less cavitation and penetration than did less saturated solutions.

The authors are pleased to acknowledge the capable assistance of Mr. Eugene Fischer for preparation of most of the sections studied. The slice technique was initially developed with the help of Miss Dorothy Harrington at the Westwood Research Laboratory.

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Studies on the Inorganic Carbon Dioxide Component of Human Enamel. II. The Effect of Acid on Enamel CO_2

MARGUERITE F. LITTLE

Eastman Dental Dispensary, Rochester, New York

The preferential removal of CO_2 by acid from bone and dentin, but not from enamel, has been inferred to mean that the CO_2 exists in an interior lattice position in enamel crystals but not in the lattice of bone and dentin.[1] Hendricks and Hill,[2] noting similar discrepancies between bone and the naturally occurring mineral francolite, suggested that enamel and francolite are composed of fused masses of individual crystallites or aggregates of crystallites containing the CO_2 entrapped on interior unavailable surfaces. They proposed that enamel CO_2 dissolution in acid would resemble that of bone if the surface area of enamel were increased from the usual 3.2 m^2/gm of powdered samples, nearer to that of bone, 60–100 m^2/gm. Posner,[3] as well as Hendricks,[2] has shown that francolite does indeed act in such a fashion. Enamel of small enough size has not heretofore been available for investigation.

In connection with studies on the CO_2 distribution in human enamel,[4] the powder ground by a diamond wheel gave surface areas ranging between 9 and 25 m^2/gm. This fortuitous range of enamel size provided an opportunity to see whether, as Hendricks and Hill propose, the CO_2 could be preferentially removed by acid. Since it was not known whether an increase in dissolution, in total acidity at a given pH, or in the amount of solid in a given solution would influence a preferential CO_2 loss, these factors (known from apatite studies to influence the loss of other constituents besides CO_2)[5, 6] were investigated. In order to make direct comparisons of this enamel with a control apatite, "L-apatite" (used by Neuman to study the nature of bone salt)[5, 6] was also included for test in one series of experiments.

EXPERIMENTAL METHODS

Enamel samples.—Two batches of small-particle-size enamel, one of 75 gm. and the other of 12 gm., were prepared by grinding the coronal surface of cleaned, freshly extracted teeth on a diamond wheel and collecting the grindings on a molecular filter,* using suction (after the method of Brudevold).[7] Care was taken to apply even pres-

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* Millipore Filter HA, Millipore Filter Corp., Bedford, Mass.

sure and to remove only enamel. The purity of each batch, that made up the pool was checked microscopically by the refractive index method of Manly and Hodge.[8] The resultant enamel (+325 mesh) samples were of fairly uniform particle size by microscopic examination and had average surface areas of 18 and 28 m^2/gm, respectively, by ethane absorption measurements.[9] The analysis of the samples, according to the methods given below, is shown in Table 1, along with the projected empirical formulas after McConnell.[10] The reason for the difference in surface area of the two samples is unknown, although the speed of the grinding wheel and the amount of pressure on the wheel may be involved. The 18 and 28 m^2/gm enamel also reacted differently in H_2O. The latter lost a measurable amount of CO_2 in water (2.40 per cent before, 2.31 per cent after), while the former gained on water treatment (2.08–2.11 per cent CO_2).

In addition, a control large-particle-size enamel (100–200 mesh) was prepared by removing debris, fillings, and roots, hollowing out all the dentin from teeth of random age, and crushing the enamel shell in a steel mill.[8] The enamel was sieved to give a sample between a 100- and 200-mesh size. It was used for a limited group of experiments.

Phosphate controls.—A sample of that "L apatite" of 68.7 m^2/gm surface area, characterized as to its chemical and physical properties by Neuman,[5, 6] was used to compare the dissolution with the enamel samples. Commercial $Ca_3(PO_4)_2$* and pure coronal dentin, ground from teeth after all the enamel had been removed, were also included for comparison.

Acid solutions.—Molar solutions of sodium and potassium acetate and sodium phosphate at pH slightly lower than 4.0, 4.5, 5.5, and 7.0 were diluted to the desired molarity just prior to use and adjusted with the hydroxide of the buffer cation to within ± 0.01 pH unit, using a pH meter.† The molarity of the solution and the volume per 100 mg. of enamel tested in the pH range 4–7 were: 0.1 M at 10, 30 and 300 ml.; 0.5 M at 10 and 300 ml.; and 0.001 to 1.0 M at 300 ml.

Sample treatment.—In most instances duplicate samples of 120 ± 0.1 mg. were used. Where large losses in weight were anticipated, the sample was increased to give a residue of at least 50 mg. The volume of solution was increased proportionately. Because of the limited amount of 28 m^2/gm enamel, the number of runs with that sample were necessarily limited. Samples of the enamel were weighed and quantitatively transferred to Pyrex flasks; after addition of buffers, the containers were sealed,† shaken at 23°–24° C. for 60 minutes, and the residue collected on weighed molecular filters§ (0.45-μ pore size) within 1 minute. The final pH was taken on this filtrate. The residue was washed twice for 10-minute periods with two equal portions of deionized, distilled water, to give a total of 100 ml wash/100 mg of original enamel. The filter, with residue, was placed on labeled watch glasses and dried in a vacuum oven at 68° C. for 1.5 hours. After weighing, the residue was removed from the filter, and samples were analyzed for CO_2, calcium, and phosphorus.

* Baker's Analyzed.

† Beckman Model G.

‡ Parafilm, Marathon Corp., Div. of American Can Co., Menasha, Wis.

§ Millipore HA, Millipore Filter Corp., Bedford, Mass.

TABLE 1

ACTUAL ANALYSIS OF GROUND ENAMEL SAMPLES

No.	Per Cent Loss 68° C. (Vacuum)	Surface Area (M^2/Gm)	Inorganic (Per Cent Dry Wt.)									Organic (Per Cent Dry Wt.)	
			Calcium		P	Ca:P (Wt.)		CO_2	Na Flame	K Flame	Mg Flame	N Per Cent*	Loss in Wt. on Ash 500° C.
			(Ca.+1.6 Mg.) EDTA	Flame		EDTA	Flame						
1...	2.12	18	36.73	36.38	17.35	2.11	2.10	2.08	0.699±.004	0.031±.001	0.210±.002	0.064	2.8
2...	1.58	28	36.80	36.44	17.60	2.09	2.07	2.40	0.70 ±.004	0.045±.001	0.21 ±.002	0.058	2.5

* Microkjeldahl.

Empirical formula (see ref. 10):

$$\text{No. 1}-(Ca, Mg)_{9\cdot3}(Na, K)_{0\cdot3}\left[\begin{matrix}(H_3O)_{0\cdot04}\\ C_{0\cdot2}\end{matrix}\right](C_{0\cdot3},\ P_{5\cdot7})O_{24}(OH)_2$$

$$\text{or} \quad (Ca, Mg, Na, K)/P = 1.692\ .$$

$$\text{No. 2}-(CaMg)_{9\cdot3}(Na, K)_{0\cdot3}\left[\begin{matrix}(H_3O)_{0\cdot4}\\ C_{0\cdot2}\end{matrix}\right](C_{0\cdot4},\ P_{5\cdot8})O_{24}(OH)_2$$

$$\text{or} \quad (Ca, Mg, Na, K)/P = 1.67\ .$$

Residue analysis.—Inorganic carbon dioxide was assessed in duplicate on 10–20-mg. ± 0.01-mg. samples of the residue by the Sobel modification[11] of the Van Slyke manometric method, using MacFadyen's reagents and factors, as previously standardized.[4] Calcium was estimated in triplicate by means of the Schwarzenback E.D.T.A. method,[12] which includes the magnesium as calcium. Periodically, absolute calcium values were obtained by flame photometry.[13] Phosphorus was determined in duplicate spectrophotometrically, by the formation of the vanado-molybdo-phosphate complex,[14] applying the type III theoretical principles of Reilley and Crawford.[15] The standard deviation of the CO_2 was 0.01, calcium 0.05, and phosphorus 0.02 as previously reported.[4]

Standardization of the method.—As established by the duplicate analysis and preliminary experiments, for any one set of conditions (solid to solution ratio, buffer, molarity, and pH), the weight losses were reproducible within 0.5 per cent. Furthermore, the weight losses for the two samples of small-particle enamel agreed within 1 per cent under identical experimental conditions. Although there was no measurable increase in total weight losses with increasing exposure to identical acid solutions for 6, 60, and 360 minutes, a decrease in the CO_2 content of the residue (0.10 per cent) occurred between 6 and 60 minutes. Since little additional change occurred in the residue CO_2 after 60 minutes, that time was used in all reported experimental work.

RESULTS

As seen in Table 2, the carbon dioxide content of every residue was lower than that of untreated enamel at all pH's, molarities, solid-solution ratios, and buffers, the residues of the 18 and 28 m^2/gm, differing in CO_2 (col. 11) as much as 40 and 44 per cent, respectively, from the untreated enamel. In contrast, the calcium and phosphorus concentrations (cols. 8 and 9) in most residues were in almost exactly the same proportions as in the untreated enamel, indicating that the losses of Ca and P from the enamel samples were stoichiometric with weight losses (col. 10). Precisely similar trends were obtained with the 28 m^2/gm enamel.

In Figure 1, the amount of residue is plotted either against the actual CO_2 content of the residue or the per cent decrease of residue CO_2 to original CO_2; the concentration of CO_2 in the residue was found to be directly related to the residue and thus decreased markedly with increasing dissolution. The amount of CO_2 loss for a given weight of residue depended on the buffer system used, with Na-PO_4 most effective, and NaAc greater than KAc. As shown in Figure 2, at the same pH and molarity, Na-PO_4 dissolves much less enamel than NaAc < KAc, particularly at high solid-solution ratios; the converse held in net pH rise. Thus, between the buffers in the order Na-PO_4 > NaAc > KAc, the over-all pH rise is inversely related to the amount of dissolution and directly related to the CO_2 loss from the residue (Figs. 1 and 2).

For any particular buffer, several interdependent factors appear to increase dissolution with a concomitant decrease in the residue CO_2 content: (1) increasing the solid-to-solution ratio, which increases the net pH rise; (2) increasing the molarity, which decreases the pH differential; and (3) lowering the pH at the same molarity, which increases the pH differential. Thus, with increased available acid, enamel acts like a buffer by increasing dissolution, the pH differential, and, importantly, the CO_2 loss from the residue.

Furthermore (Fig. 3), ground enamel dissolution was identical with Neuman's "L-

TABLE 2*

Effect of Various Acid Buffers on Enamel of 18 M^2/Gm Surface Area

Expt. No.	Solution Composition						Residue Analysis (Per Cent)			Weight Loss (Mg/100 Mg)	Per Cent Decrease in CO_2 of Residue
	pH		Composition		Molarity	Ml. Soln. per 100 mg. Solid	CO_2	Ca	P		
	Initial	Final	3. Cation	4. Anion							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
							2.08 (Original Analysis)	36.7	17.4		
1....	4.00	4.58	Na	Ac	0.01	300	1.25	36.7	17.4	83.0	39.9
2....	4.00	5.60			0.001	300	1.74	36.3	17.9	42.0	16.4
3....	4.04	4.50			0.1	30	1.72	.....	.....	43.1	17.3
4....	4.04	4.62			0.1	10	1.68	.....	17.3	24.9	19.2
5....	4.03	4.18			0.5	10	1.75	37.2	17.9	42.1	15.9
6....	4.00	4.58	K	Ac	0.1	30	1.81	.....	.....	42.8	13.0
7....	4.00	4.61			0.1	10	1.84	.....	.....	27.6	11.5
8....	4.00	4.41			0.5	10	1.69	37.6	17.8	51.5	18.8
9....	4.49	4.54	Na	Ac	0.1	300	.....	.....	.....	87.2	.....
10....	4.51	4.98			0.01	300	1.57	37.1	17.9	67.8	24.5
11....	4.50	5.60			0.001	300	1.78	.....	.....	16.4	14.4
12....	4.56	4.83			0.1	30	1.77	36.3	17.9	35.0	14.9
13....	4.54	4.90			0.1	10	1.88	.....	.....	18.0	9.6
14....	4.54	4.60			0.5	10	1.77	36.8	17.2	33.0	14.9
15....	4.50	4.50	K	Ac	0.1	300	.....	.....	.....	90.1	.....
16....	4.54	4.82			0.01	300	1.61	36.7	17.3	66.2	22.6
17....	4.52	5.28			0.001	300	1.64	.....	.....	16.2	21.2
18....	4.50	4.61			0.1	30	1.78	.....	.....	35.1	14.4
19....	4.50	4.70			0.1	10	1.98	.....	.....	21.9	4.8
20....	4.46	4.54			0.5	10	1.87	37.0	17.2	29.2	10.1
21....	4.50	4.56	Na	PO_4	1.0	300	.....	.....	.....	49.2	.....
22....	4.50	4.91			0.1	300	1.80	.....	.....	26.6	13.5
23....	4.50	5.61			0.01	300	1.89	36.9	17.6	9.1	9.1
24....	4.50	5.61			0.001	300	1.99	.....	.....	6.3	4.3
25....	4.50	5.30			0.1	30	1.82	.....	.....	5.4	12.5
26....	4.51	5.32			0.1	10	1.91	.....	.....	1.9	8.2
27....	4.50	4.85			0.5	10	1.94	36.5	18.1	5.7	6.7
28....	4.50	5.00	K	Phthalate	0.01	300	1.66	35.6	17.4	46.6	20.2
29....	4.54	5.81			0.001	300	1.66	.....	.....	19.1	20.2
30....	4.50	4.52			0.5	10	1.86	36.7	17.2	36.5	10.6
31....	5.46	5.50	Na	Ac	1.0	300	1.51	.....	.....	68.0	27.4
32....	5.50	5.50			0.5	300	1.51	.....	.....	65.1	27.4
33....	5.50	5.60			0.1	300	1.61	.....	.....	49.5	22.6
34....	5.50	6.10			0.01	300	1.81	37.1	17.9	25.1	13.0
35....	5.50	6.80			0.001	300	1.97	.....	.....	13.3	5.3
36....	5.46	5.70			0.1	30	1.94	.....	.....	13.7	6.7
37....	5.55	5.80			0.1	10	2.00	.....	.....	5.9	3.8
	5.50	5.60			0.5	10	1.91	36.8	17.9	11.1	8.2
38....	5.50	5.52			1.0	10	.....	.....	.....	15.5	.....
39....	5.50	5.50	K	Ac	0.1	300	1.61	.....	.....	56.7	22.6
40....	5.52	5.70			0.01	300	.....	.....	.....	23.6	.....
41....	5.60	5.83			0.001	300	.....	.....	.....	6.5	.....
42....	5.50	5.64			0.1	30	1.88	.....	.....	15.8	9.6
43....	5.50	5.78			0.1	10	1.97	.....	.....	7.2	5.3
44....	5.50	5.50			0.5	10	2.05	36.9	17.6	16.8	1.4

* Each number is the mean of two determinations.

TABLE 2—*Continued*

Expt. No.	Solution Composition						Residue Analysis (Per Cent)			Weight Loss (Mg/100 Mg)	Per Cent Decrease in CO_2 of Residue
	pH		Composition		Molarity	Ml. Soln. per 100 mg. Solid	CO_2	Ca	P		
	Initial	Final	3. Cation	4. Anion							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
45....	5.52	5.58	Na	PO_4	0.1	300	1.87	.....	.....	11.8	10.1
46....	5.52	5.64			0.1	30	1.93	.....	.....	2.6	7.2
47....	5.52	5.71			0.1	10	1.82	.....	.....	0.6	12.5
48....	5.51	5.55			0.5	10	1.92	37.1	17.8	2.3	7.7
49....	6.94	6.96	Na	PO_4	1.0	300	1.92	.....	.....	5.0	7.7
50....	7.00	7.00			0.1	300	1.93	.....	.....	5.3	7.2
51....	6.92	7.00			0.01	300	1.96	36.6	17.6	4.4	5.8
52....	7.00	7.04			0.1	30	2.00	.....	.....	2.0	3.8
53....	7.00	7.00			0.1	10	1.98	.....	.....	0.3	4.8
54....	6.98	6.97			0.5	10	2.00	.....	.....	0.3	3.8

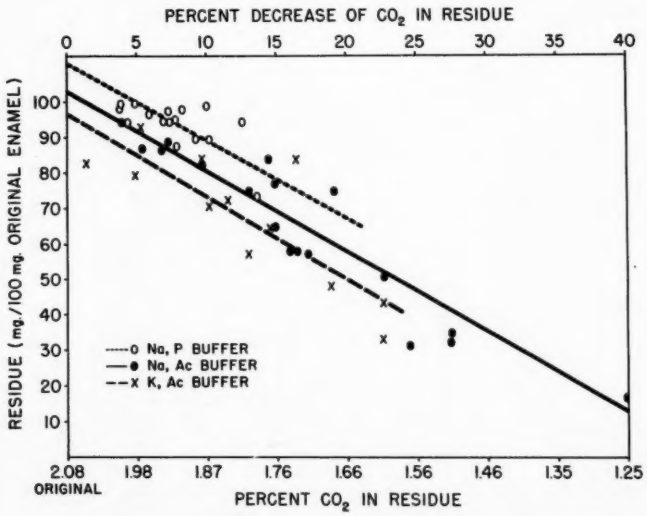

FIG. 1.—The increasing preferential CO_2 loss from, and the corresponding decreasing CO_2 content of, the residue with increased dissolution of 18 m^2/gm enamel in sodium acetate, potassium acetate, and sodium phosphate buffers, regardless of pH, molarity, etc., is shown. The buffers, in order of effectiveness in causing a CO_2 loss from a given weight of residue, were sodium phosphate, sodium acetate, and potassium acetate.

apatite" and commercial $Ca_3(PO_4)_2$ over the pH range 3.5–6.5, whether initial or final pH's were considered. Ground dentin losses were somewhat higher, and, as seen by comparing Tables 3 and 2, crushed 100–200-mesh enamel not only failed to reach equilibrium in the experimental hour interval but failed, as expected,[16] to show preferential CO_2 losses except under the most extreme conditions. A minimum of 5 hours

was required to bring weight losses of the 100–200-mesh enamel to that used in this study.

DISCUSSION

Thus, though the residue CO_2 content is directly related to the amount of residue, the Ca and P contents are not. Experimentally in this enamel, the CO_2 could not be removed completely without destroying the structure. Therefore, though part of the enamel CO_2 was preferentially removable, part was not.

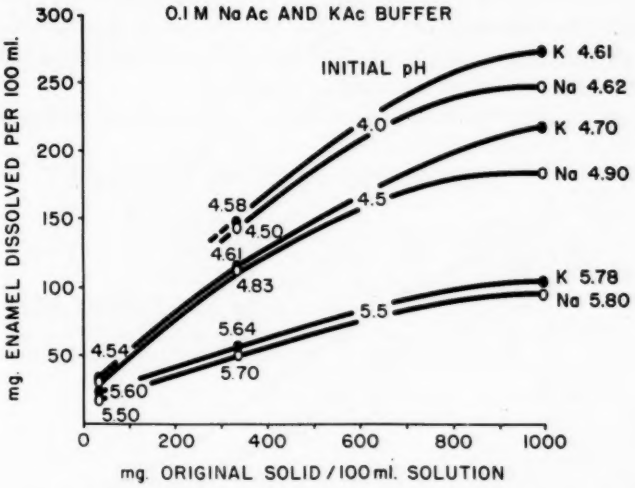

FIG. 2.—Increasing the amount of enamel in 100 ml. of 0.1 M sodium and potassium acetate buffers at pH 4.0, 4.5, and 5.5 increased dissolution. The final pH's (as shown alongside the individual points) increased with increasing dissolution. Potassium acetate buffer increased dissolution more than sodium acetate, yet caused a lower net pH rise, especially at high solid/solution ratio.

TABLE 3

EFFECT OF SODIUM ACETATE BUFFER ON 200-MESH CRUSHED ENAMEL (1 HOUR)

EXPT.*	SOLUTION				RESIDUE ANALYSIS (PER CENT)			WT. LOSS (MG/ 100 MG)	PER CENT LOSS TOTAL CO_2
	pH		Molarity	100 Mg/ ml.	Ca	P	CO_2		
	Initial	Final							
					38.2	17.7 (Original)	2.58		
78.....	4.00	4.29	0.1	10	38.2	17.6	2.56	14.4	15.1
79.....	4.00	4.30	0.1	30	38.2	17.7	2.52	34.8	36.3
80.....	4.00	4.07	0.1	300	38.2	17.6	2.40	54.2	57.4
81.....	4.00	4.30	0.01	300	38.1	17.8	2.55	23.1	24.0
82.....	4.47	4.57	0.1	10	38.2	17.6	2.58	9.1	9.1
83.....	4.47	4.57	0.1	30	38.3	17.7	2.56	20.4	21.1
84.....	4.47	4.50	0.1	300	38.1	17.7	2.47	42.5	44.9

* Mean of three determinations.

Nevertheless, a direct relationship does exist between non-stoichiometric CO_2 removal and stoichiometric removal of Ca and P. The relationship, based on total percentage weight losses—which are equivalent to total per cent Ca or P losses—is independent of experimental conditions such as pH, molarity, etc. Figure 4 (on 18 m^2/gm) and Figure 5 (on 28 m^2/gm) enamel show that the total weight (or Ca and P total losses) is directly proportional to total disproportionate CO_2 loss. The mathematical form is $Y = a\,X - c$, where Y is per cent total weight, per cent total Ca, or per cent total P lost; a, the slope, is 0.978; X is total per cent CO_2 lost; and c, the disproportionate CO_2 loss, is equal to 8 per cent for 18 m^2/gm and 12 per cent for 28 m^2/gm enamel. The coefficient of correlation is 0.997, with experimental limits between 0 and 83 per cent weight losses. The 100–200 crushed enamel has a constant disproportionate CO_2 loss (c) of less than 1 per cent.

Since preferential removal of CO_2 from this enamel occurred with neutral or acid

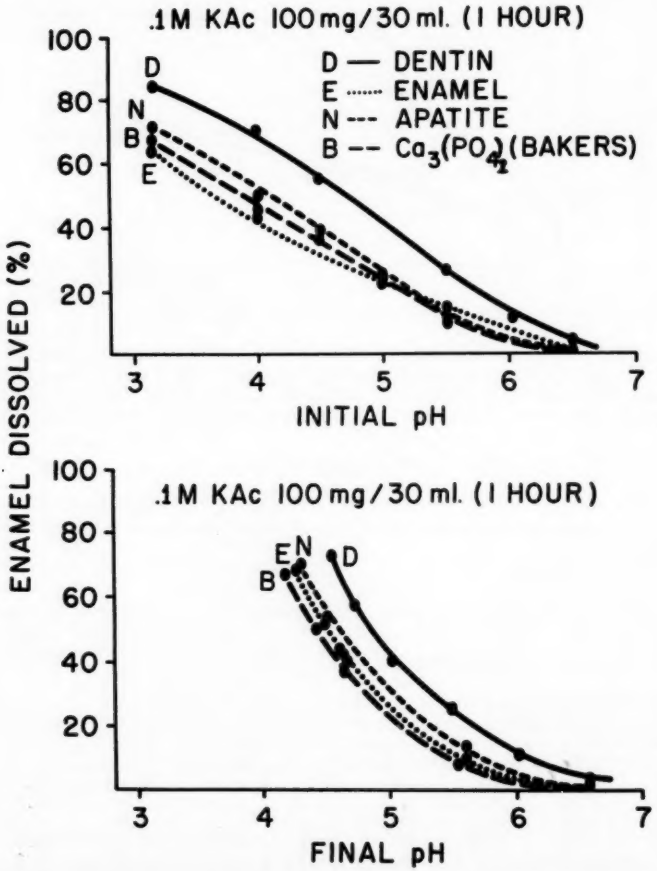

FIG. 3.—Similar amounts of enamel (18 m^2/gm), Neuman's "L-apatite" (68.7 m^2/gm), and commercial $Ca_3(PO_4)_2$ dissolved in 0.1 M potassium acetate buffer (100 mg, 30 ml.) over an initial pH range of 3.0–6.5 or a final pH of 4.0–6.8. Ground dentin showed a higher dissolution and a greater pH rise under the same conditions.

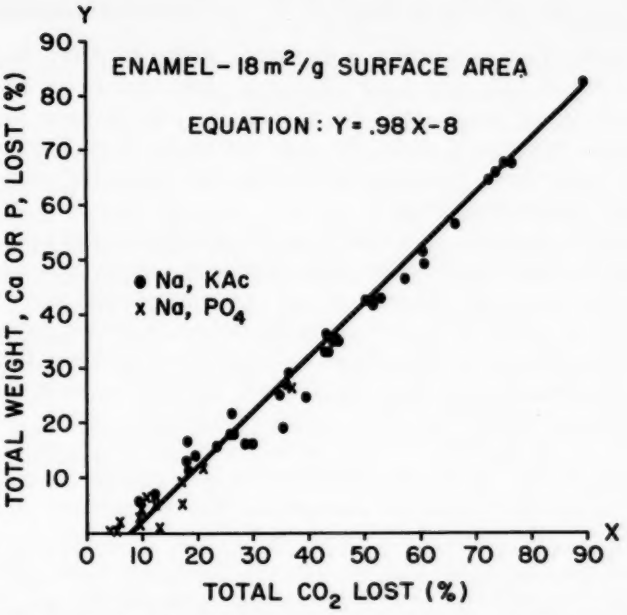

FIG. 4.—The total removal of CO_2 from 18 m^2/gm enamel was 8 per cent greater than either total weight, calcium or phosphorus, regardless of dissolution, buffer molarity, etc.

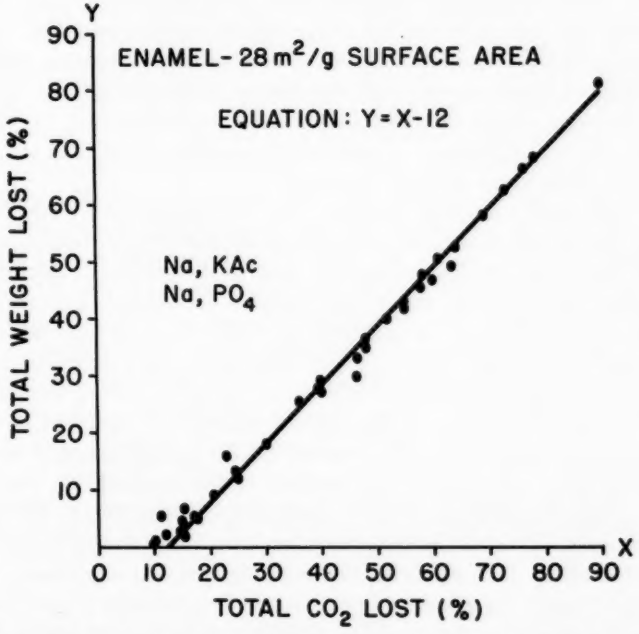

FIG. 5.—The total removal of CO_2 from 28 m^2/gm enamel was 12 per cent greater than either total weight, calcium or phosphorus, regardless of dissolution, buffer molarity, etc. (each point one determination).

solutions containing Na^+ and/or PO_4, a heteroionic exchange of PO_4, H_3O^+, or Na^+ for surface-contained CO_2 may occur similar to that reported in bone and apatites.[6, 17–20] This is not unreasonable because this enamel compares favorably with apatites in other respects: it obeys the same factors as those influencing apatite loss,[5, 6, 18] and it dissolves almost identically with that "L-apatite" used by Neuman in establishing heteroionic exchange.[5, 6]

The amount of exchange of an ion in an apatite is a function of the available surface area.[6] The availability of surface components, in turn, is enhanced by small-particle-size dimensions, since the ratio of area to volume of solid particles of similar shape is inversely proportional to their linear dimensions. Normally, bone and enamel particles differ in surface area, since the small dispersed crystallites of bone invariably give it a remarkably large surface area—100–200 m^2/gm[6, 21]—while the large compacted crystallites of enamel[22] give, in contrast, a surface area of only between 1 and 3 m^2/gm.[2, 6] Even when enamel is cleaved, as in this study, giving a surface area closer to that of bone or apatites, neither the dimensions of bone crystals nor the surface area were reached. Hence only when the enamel was almost completely dissolved, with each remaining particle consequently diminished in size (probably equally on each dimension), did this enamel simulate the reported 50 per cent excess removal of CO_2 normally found in bone or apatites.[2, 23] Yet increasing the surface area of enamel by further decreasing its initial particle size will no doubt increase the preferential CO_2 removal to approximate that of bone and apatites.[2, 23]

These findings support the suggestions of Hendricks and Hill[2] that preferential removal of CO_2 would occur if the enamel particle, as in these experiments, is sufficiently small. However, the fact that (*a*) the CO_2 content of the residue decreased directly and cumulatively with decreasing amounts of residue; (*b*) the excess removal of CO_2 on acid treatment for any given weight loss was a fixed constant, increasing with increasing surface area of the samples; and (*c*) the possibility that the mechanism of removal is heteroionic exchange[5, 6] suggested the following alternative to the entrapped surface and void hypothesis of those authors.[2]

Assuming that in all carbonate-apatites, including enamel and bone, the carbonate is present as part of the structure and is randomly and evenly distributed in accordance with the empirical formula (which also denotes the unit cell), then in bone (with 4 per cent CO_2) every unit cell would have one CO_3 group, and in enamel (with 2 per cent CO_2) every other unit cell would have one CO_3 group. In any particle a portion of the CO_2 may be assumed to be preferentially removable, probably by H_3O^+ replacement, since a portion would invariably be available on the surface, e.g., in a surface unit cell. A monomolecular surface layer would then be deficient in CO_2, though intact in other constituents. The CO_2 content of the treated particle would then be lower than originally, and equal to the product of the original CO_2 content and the ratio of the monomolecular surface layers (unit cell) lacking CO_2 to the total number of layers (unit cells). On small enough particles, the change would be measurable and inversely related to the decrease in the linear dimensions of the particles, if they were of similar shape. Hence the amount of preferential CO_2 removal in any apatite should be a constant, dependent on the available surface to total volume and independent of the amount of CO_2 in the material. Based on this concept, the ratio of surface cells in the 18 m^2/gm enamel to total cells would be 8 per cent, indicating an approximate average thickness of 24 "unit cells" ($2/24 = 8$ per cent) and the 28 m^2/gm with a

constant of 12 per cent—an average thickness of approximately 16 "unit cells" (2/16 = 12 per cent). Since bone reportedly has a constant of 40–50 per cent according to Logan and Taylor,[23] one can predict a thickness of approximately 3–5 unit cells—a not unreasonable figure.[21] Similarly, from this hypothesis, a normal maximal preferential removal of CO_2 in any carbonate-apatite would be 66–75 per cent, indicating a thickness of 3–4 unit cells; and only in the unlikely situation where a carbonate-apatite lattice comprised only surface, containing unit cells, would all the CO_2 be removable, preferentially, without completely destroying the structure. Based on these considerations, it is possible that the CO_2 may be an integral part of the apatite lattice, albeit, when in the surface, a labile one.

Preferential removal of CO_2 would thus appear to be a natural phenomenon in enamel as well as bone[23] and apatite,[2, 23] and the lack of preferential removal of CO_2 from enamel can no longer be used to support a different mineral structure for enamel, bone, or carbonate-apatites.[1, 2, 6]

SUMMARY

When ground enamel samples with surface areas of 18 and 28 m^2/gm (by ethane absorption measurement) were treated with acetate and phosphate buffers of pH 4–7 over a range of molarity, and solid-to-solution ratios, the residue was found to have up to 40–44 per cent less CO_2 than the original enamel. Under similar experimental conditions, enamel of larger particle size (100–200 mesh) failed to lose CO_2 in detectable amounts. The buffers were sodium phosphate, sodium acetate, and potassium acetate, in the order of their effectiveness in increasing the removal of CO_2 from the residue (with less dissolution but a greater net rise in pH). This was interpreted to mean that the exchange of H_3O^+ for CO_2 in the enamel was increased by sodium and by phosphate ions. However, in the same buffer, increasing the dissolution increased the CO_2 losses from the residue. The factors which increased the dissolution were original pH, pH rise with increasing solid-to-solution ratios, and molarity. A constant preferential removal of CO_2 was found that was statistically independent of the amount of dissolution or the factors influencing it but was a function of the available surface. The 18 m^2/gm gave a constant of 8 per cent; the 28 m^2/gm, a constant of 12 per cent; while the 100–200 mesh gave a constant of <1. On the basis of these results, it was proposed that the CO_2 in enamel is statistically in an even distribution in the unit cells of the apatite lattice and that the phenomenon of a constant preferential removal in apatites is a reflection of the removal of replaceable or labile CO_2 by H_3O^+, phosphorus, or sodium from surface unit cells and is a direct function of the ratio of surface area to total volume.

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Studies on the Carbon Dioxide Component of Human Enamel. III. The Effect of Neutral and Acid Fluoride

MARGUERITE F. LITTLE and JANET ROWLEY
Eastman Dental Dispensary, Rochester, New York

Since the mechanism of fluoride interaction with enamel CO_2, as well as apatites, was of fundamental interest,[1, 2] the effect of neutral and acid fluoride on the CO_2 portion of small-particle-size enamel, previously characterized[3] as to its reactions with acid buffers, was investigated. Enamel of larger particle size (and correspondingly smaller surface area) has been investigated by McCann[1, 4] for reactions occurring in neutral fluoride solutions in the presence and absence of phosphates. McCann suggested that one of the reactions of fluoride may be the removal of CO_2. This study was conducted to determine whether a smaller-particle-sized enamel would allow observation of even more marked effect on the CO_2 component than it did with acid or phosphate alone.[3]

MATERIALS AND METHODS

The pooled sample of enamel of 18 m^2/gm surface area,[3] previously studied for changes in chemical composition after treatment with acetate and phosphate buffers over a wide range of molarity, pH, and solid-solution ratios, was used for this study. In order to determine dissolution, samples of the enamel were treated by incorporating the following three types of fluoride solutions of varying concentrations from 0 to 9,050 p.p.m. in the dissolving medium: (1) fluoride in water, (2) fluoride in neutral 0.5 M phosphate buffer, and (3) fluoride in pH 4.5, 0.5 M acetate, and phosphate buffers. With 1 and 2 the amount of enamel per volume of solution was 100 mg. in 10, 30, and 300 ml.; with 3, only the highest solid-to-solution ratio (100 mg/10 ml) was used.

A stock solution of sodium fluoride containing 10,000 p.p.m. fluoride, prepared from reagent-grade sodium fluoride, was diluted just prior to use to obtain 1, 10, 100, 1,000 up to 9,050 p.p.m. fluoride ion in the final solution. For acidulated solutions with fluoride, portions of stock fluoride were added to the required volume of molar buffer at a lower pH, the solution brought nearly to volume, adjusted quickly to the desired pH, and brought up to volume. For high concentrations of fluoride (9,050 p.p.m.), weighed amounts of sodium fluoride were added to appropriate molar buffer solutions, near-dilution was made, and the pH adjusted with a minimum exposure of the elec-

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trodes, in order to prevent etching. Polyethylene containers were used in all fluoride operations.

Stock molar solutions of sodium acetate, potassium acetate, and sodium phosphate at pH 4.5 and sodium phosphate at pH 7.0 were diluted to the desired molarity just prior to use and adjusted with the hydroxide of the buffer cation to within ±0.01 of the desired pH.

Sample treatment.—Sample treatment was identical with that reported previously, where 120-mg. samples of the pooled enamel were weighed to ±0.1 mg. and transferred to polyethylene containers. After addition of the test solution, the containers were sealed* and shaken for 1 hour at 23°–24° C. The sample was collected on weighed molecular filters,† washed with two equal portions of water for a total of

TABLE 1

EFFECT OF TREATING ENAMEL WITH UNBUFFERED SOLUTIONS OF INCREASING FLUORIDE CONCENTRATION

p.p.m. F as NaF	Solution				Residue Analysis						Loss as Per Cent of Total						
	pH				Per Cent CO_2 in		Per Cent P in		Per Cent Ca in		Weight			CO_2		P 300*	Ca 300*
	Initial	Final 300*	Final 30*	Final 10*	300*	10*	300*	10*	300*	10*	300*	30*	10*	300*	10*		
O (H_2O alone)	6.2	7.0	7.0	7.0	2.14	2.10	17.4	17.6	36.8	36.7	3.3	...	2.2	0.2	1.2	3.3	3.3
1.....	5.8	7.8	7.6	7.6	2.04	2.15	17.4	17.4	35.8	36.4	1.6	2.0	1.0	3.5	+2.3	1.6	1.6
10.....	6.2	7.3	7.7	7.8	2.06	.....	17.7	17.2	36.7	35.6	3.6	1.8	1.8	4.5	.....	3.6	3.6
100.....	6.5	7.7	8.3	8.9	2.08	2.14	17.4	17.2	38.1	.....	2.9	1.0	1.6	2.9	+1.2	2.9	0.0
1,000.....	6.7	8.0	9.0	9.3	2.10	2.13	17.5	17.5	36.1	37.1	2.8	0.9	0.8	1.9	+1.6	2.8	4.6
2,000.....	7.0	8.6	9.3	9.5	2.04	2.05	17.2	17.3	36.4	36.7	1.6	0.4	1.2	3.5	2.6	2.9	2.5
4,500.....	7.0	8.9	9.5	9.4	1.94	2.13	16.8	17.5	36.7	37.1	2.8	1.2	2.3	9.3	0.0	6.2	2.8
9,050.....	7.1	9.1	9.7	9.7	1.75	2.14	15.4	17.8	37.2	37.3	4.3	2.3	1.5	19.5	+1.3	14.9	3.0
Original.....					2.08		17.4		36.7								

* Ml solution/100 mg solid.

100 ml of wash/100 mg original enamel, and dried to constant weight at 68° C. in a vacuum oven. Final pH's were taken on the filtrate before the wash. Samples of the residue were analyzed for CO_2, calcium, and phosphorus. The analyses were carried out on residues as previously described.[3]

RESULTS

Fluoride in water.—The effect of fluoride (as NaF) from 0 to 9,050 p.p.m. in water on the small-particle-size enamel is shown in Table 1. From the analysis of the residue after treatment with the fluoride solution at high solid-to-solution ratios (100 mg/10 ml), it seemed that an increase in the CO_2 content and possibly in the phosphorus and calcium content may have occurred. However, a calculation of total losses showed

* Parafilm, Will Corporation, Rochester, N.Y.

† Millipore HA, Millipore Filter Corp., Bedford, Mass.

that, despite an approximate 2 per cent weight loss, there was little calcium, phosphorus, or CO_2 loss with increasing fluoride concentration. However, with both high and low solid-to-solution ratios, the pH of the solution rose with increasing fluoride concentration—the higher the ratios, the greater the rise.

With low solid-solution ratios (100 mg/300 ml) at fluoride levels from 1 to 1,000 p.p.m., the CO_2 content of the residue was similar to that of the untreated enamel, while, above 1,000 p.p.m., the CO_2 in the residue decreased with increasing fluoride concentration. At 9,050 p.p.m. fluoride, 20 per cent of the total CO_2 was removed, while only 4.3 per cent of the weight was lost. Analysis of the components other than CO_2 showed that the calcium of the residue increased slightly, while the phosphorus decreased markedly above 2,000 p.p.m. fluoride. From large phosphate losses as compared with calcium losses, large conversion[4] to calcium fluoride must have occurred at 9,050 p.p.m.; total phosphate loss was less, however, than the total CO_2 loss.

TABLE 2

INCORPORATION OF FLUORIDE IN PH 7.0 PHOSPHATE BUFFER AT HIGH AND LOW SOLID TO SOLUTION RATIOS

F	High (100 Mg Solid/Ml Solution)					Low (100 Mg Solid/Ml Solution)				
	10 Ml (0.5 Molar)					300 Ml (0.1 Molar)				
	Residue Analysis Per Cent Dry Weight			Wt. Loss	Per Cent Loss Total CO_2	Residue Analysis Per Cent Dry Weight			Wt. Loss	Per Cent Loss Total CO_2
	Ca	P	CO_2			Ca	P	CO_2		
0...	36.5	18.0	1.98	0.3	5.1	36.7	17.5	1.94	5.3	11.7
1...	38.0	17.6	2.05	2.2	3.6	36.8	17.6	1.90	5.7	13.9
10...	37.0	17.6	1.96	2.6	8.2	36.8	17.5	1.83	5.1	16.5
100...	36.6	17.5	2.00	0.8	4.6	36.6	17.5	1.83	3.2	14.9
1,000...	37.0	17.4	1.97	0.0	5.3	37.2	17.4	1.85	3.6	14.3
9,050...	37.2	16.9	1.95	3.2	9.3	36.9	17.2	1.77	3.0	17.5

Fluoride addition to neutral phosphate buffers.—The effect of treating small-particle-size enamel with fluoridated neutral phosphate buffer at high and low solid-solution ratios is shown in Table 2. At high ratios (100 mg/10 ml), the loss of CO_2 is less than that found with low ratios (100/300 ml), regardless of the presence or absence of fluoride. Only at 9,050 p.p.m. of fluoride was the removal of CO_2 from the residue slightly increased. However, with low solid-to-solution ratios (100/300 ml), the CO_2 losses increased additively with increasing concentrations of fluoride until, at 9,050 p.p.m., losses were similar in either buffered or (Table 1) unbuffered solutions. Yet, no losses of calcium and phosphorus occurred on treating enamel with up to 9,050 p.p.m. fluoride. However, with high solid-to-solution ratios, at 9,050 p.p.m. concentration, the increased phosphate and weight losses suggest that a slight double decomposition to calcium fluoride may have occurred.[1, 4]

Acidulated fluoride solutions.—In order to minimize the above effect of fluoride, that solid-solution ratio which had the least interaction with the enamel (100 mg/10

ml) was used in acid buffers. For pH stability, only pH 4.5, 0.5 M buffers were used, since the pH increased only 0.10 in acetate and 0.05 pH units in phosphate buffers during the treatment.

As seen in Tables 3 and 4, while fluoride above 1 p.p.m. decreased weight losses markedly in acetate and slightly in phosphate buffers, at 9,050 p.p.m. the weight losses in phosphate and acetate buffers were equal. However, weight losses in fluoride solution are not a measure of the losses of calcium and phosphate or CO_2. In the acid buffers, up to 100 p.p.m. fluoride inhibited, while, above 100 p.p.m., fluoride augmented CO_2 losses from the enamel.

Non-stoichiometric removal of CO_2 occurs in all the acid fluoride buffers, with the total CO_2 losses exceeding the total weight losses by at least the 6–8 per cent previously reported for this enamel in the buffer alone.[3] Above 1,000 p.p.m., CO_2 losses in-

TABLE 3

PER CENT CA, P, AND CO_2 IN RESIDUE AFTER TREATMENT OF 18 M^2/GM ENAMEL WITH FLUORIDE IN PH 4.5, 0.5 M BUFFERS (SOLID-SOLUTION RATIO 100 MG/10 CC)

P.P.M. F ADDED	BUFFER COMPOSITION															
	Potassium Acetate				Sodium Acetate				Sodium Phosphate				Potassium Phthalate			
	Ca	P	Ca:P (Wt.)	CO_2	Ca	P	Ca:P (Wt.)	CO_2	Ca	P	Ca:P (Wt.)	CO_2	Ca	P	Ca:P (Wt.)	CO_2
Control.	36.7	17.4	2.11	2.08	36.7	17.4	2.11	2.08	36.7	17.4	2.11	2.08				
0 (acid alone).	37.0	17.2	2.15	1.85	36.8	17.2	2.14	1.77	36.5	18.1	2.02	1.94	36.7	17.3	2.12	1.86
1..	35.7	17.3	2.06	1.87	.....	.....	.....	.....	36.7	17.4	2.11	1.93	.....	.....	.....	.....
10..	42.4	17.5	2.42	1.94	40.7	17.6	2.31	1.98	36.9	17.4	2.12	1.98	.....	.....	.....	.....
100..	40.0	16.5	2.42	1.86	.....	.....	.....	.....	37.7	17.3	2.18	1.88	.....	.....	.....	.....
1,000..	38.4	15.0	2.56	1.59	38.4	14.5	2.64	1.27	37.8	17.1	2.21	1.70	.....	.....	.....	.....
9,050..	42.5	9.8	4.34	0.78	42.2	10.7	3.94	0.89	41.6	11.4	3.65	1.34	41.8	13.2	3.17	1.36

TABLE 4

EFFECT OF FLUORIDE INCORPORATION IN ACETATE AND PHOSPHATE BUFFERS (PH 4.5) ON ENAMEL*

BUFFER P.P.M. F AS NAF	LOSS AS PER CENT OF TOTAL COMPONENT											
	Potassium Acetate pH 4.5				Sodium Acetate pH 4.5				Sodium Phosphate pH 4.5			
	Wt.	Ca	P	CO_2	Wt.	Ca	P	CO_2	Wt.	Ca	P	CO_2
0 (acid alone)..	29.2	28.6	30.0	37.0	33.0	32.3	33.8	43.0	5.7	6.2	1.9	12.0
1.....	32.0	33.9	32.4	38.9	.....	.....	.....	.....	7.0	7.0	7.0	13.7
10.....	22.6	10.6	22.2	27.8	21.9	13.4	21.0	25.7	5.8	5.3	5.8	10.3
100.....	15.3	7.7	19.7	24.3	.....	.....	.....	.....	3.8	1.2	4.4	13.0
1,000.....	15.8	11.9	27.4	35.6	12.6	8.6	27.2	46.6	4.7	1.8	6.4	22.1
9,050.....	13.5	+ 0.2	51.3	67.6	12.0	+ 1.0	45.9	62.4	12.3	0.5	42.5	43.4

* Solid/solution ratio of 100 mg/10 cc, molarity 0.5.

creased sharply, with between 40 and 60 per cent of the total CO_2 being removed for a 13 per cent weight loss. Losses of phosphate, although less, paralleled CO_2 losses above 10 p.p.m. in acetate and above 100 p.p.m. in phosphate buffers. In contrast, the calcium losses decreased steadily with increasing fluoride concentrations above 1 p.p.m. and inversely with the Ca/P ratios. Thus at 10–100 p.p.m. fluoride in acid acetate buffers, one portion of phosphate must be removed for each portion of CO_2 preferentially lost over and above the normal 8 per cent occurring in acid alone.[3] Since CaF_2 formation equivalent to the phosphate losses is patently impossible with the amount of sodium fluoride present, fluoroapatite conversion of the enamel is highly probable, with some phosphate either being replaced by water[5] or left "vacant."[6] At 1,000 and 9,050 p.p.m. fluoride, most of the phosphate lost in excess of calcium can be accounted for by the formation of CaF_2,[1, 4] and the losses of CO_2 from the remaining residue not attributable to CaF_2 formation appear to be at least double that found in non-fluoride solutions. It is interesting that, as at lower concentrations of fluoride, some additional losses of phosphate appear to accompany the portion of CO_2 lost in excess of that normal in the acid alone, and phosphate as the buffer anion inhibits this action.

DISCUSSION

The results indicate that, under specific conditions, sodium fluoride either alone or in acid will effect preferential removal of large excesses of CO_2 from enamel of small-particle size. The preceding study[3] suggested that smaller amounts of CO_2 can be preferentially removed or exchanged in vitro by either phosphate or acid from the same enamel. Hence it was not unexpected that fluoride addition to neutral phosphate buffer at low solid-solution ratios increased the CO_2 removal additively at low fluoride concentrations and equally well at high fluoride concentrations. Although fluoride alone at high solid-solution ratios tends not to remove CO_2 preferentially, when it was incorporated in concentration at or above 10 p.p.m. in acid acetate buffers it augmented the previously established[3] constant preferential losses of CO_2 in acid. Although acidic phosphate buffer interfered with excessively large removals of CO_2 at most fluoride concentrations, it did not stop the massive conversion through double decomposition[1, 4] of apatite to calcium fluoride at the high fluoride concentrations tested; more actual destruction of apatite would appear to occur with acid 2 per cent sodium fluoride than with acid alone. Not only at the high but also at the low concentrations of fluoride, the losses of CO_2 and lower losses of phosphate, over and above that attributable to CaF_2, further suggest that marked remodeling of the residual enamel occurs in the absence of phosphate as the buffer anion. In fact, chemically, one can suggest with McCann[1, 4] the formation of an isomorphic variant[5, 6] of fluoroapatite under these conditions.

Neuman previously suggested that in apatite and bone salt the small un-ionized acid fluoride penetrates beyond the exposed crystallite surface and removes CO_2 from the interior of the crystal.[2] About 8 per cent of the total CO_2 is preferentially removable from this particular sample of enamel in acid alone; Little[3] suggested that the removable CO_2 was in the exposed surface of the particles. Since in the present study at least double that amount of total CO_2 was preferentially removed in the presence of acid fluoride, the acid fluoride (HF) must penetrate the subsurface and eliminate additional CO_2 groups. Since common phosphate ions suppressed both excessive CO_2 and

PO_4 removal, it is possible that the CO_2 loss from the subsurface usually involves removal of an intervening phosphate. This suggestion is in keeping with the previous proposal of Little[3] that the CO_2 of enamel is in a statistically even distribution in the "apatite" lattice, where phosphate would, of necessity, occupy an intervening space. It is interesting that McCann reports excess phosphate removal from large-particle enamel under conditions of neutral fluoride treatment when CaF_2 would not form; whether excess CO_2 was also removed would not be detectable with his size of particles,[1, 4] although his results indicated that it was.

In view of the reduction in dissolution, the possible deeper penetration, and the marked production of the more desirable fluoroapatite as opposed to CaF_2 in low acid fluoride solution (10–100 p.p.m.), the possible therapeutic value of low concentrations of acid sodium fluoride in vivo deserves further investigation. In fact, one might suggest that the purported greater effectiveness of SnF_2[7] over NaF as an enamel protective agent may be related to the acidic properties[8] of the former.

SUMMARY

Addition of more than 1 p.p.m. fluoride to acid acetate buffers increased the amount of CO_2 preferentially removed by acid or fluoride alone from enamel of very small-particle size. When calcium fluoride was not a major reaction product, almost equivalent portions of phosphorus accompanied that part of CO_2 removal in excess of the CO_2 normally removed preferentially in acid. Phosphate buffer suppressed this effect. It was theorized that acid fluoride penetrated below the exposed surface to effect, in some manner, the removal of subsurface CO_2. Although marked conversion to calcium fluoride occurred with acidulated 2 per cent sodium fluoride, in the absence of phosphate buffer more than 20 per cent of the CO_2 in the remaining unconverted enamel appeared to be preferentially removed.

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Mast-Cell Density in Diphenylhydantoin Sodium Gingival Hyperplasia

C. O. DUMMETT, T. E. BOLDEN, and J. C. ASHHURST

Veterans Administration Hospital, Tuskegee, Alabama, and Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey

Mast cells have been described in normal gingivae of the monkey and humans. They have also been described in acute necrotizing gingivitis,[1] chronic marginal gingivitis,[2] and pregnancy gingivitis[2] (Table 1). The recorded observations on mast-cell density in these tissues are quite variable. Wislocki and Sognnaes[3] reported 30–60 mast cells in the ground substance of the normal gingiva of a young child. Carranza and Cabrini[1] reported 1.9 mast cells per field, Stella[2] indicated that mast cells were "numerous" in normal gingiva, while Dewar[4] found a mast-cell concentration of 18.3 (S.D. 14.3) per square millimeter of tissue. Stella[2] also reported that mast cells were more numerous in pregnancy gingivitis than in normal gingiva. Carranza and Cabrini[1] showed either a decrease in number or no change in the number of mast cells in acute and chronic gingivitis. Dewar demonstrated an increase in mast-cell density in inflammed gingival tissue.[4]

To extend these observations, quantitative determinations have been made of the mast cell in sections of gingiva diagnosed clinically and histologically as (1) reactive or (2) normal. This report is limited to the mast-cell density in sections of gingivae of epileptic patients maintained on protracted courses of diphenylhydantoin sodium therapy.[5]

MATERIALS AND METHODS

Sections of attached and free gingivae were taken from the mouths of nine epileptic patients, inmates of the Veterans Administration Hospital, Tuskegee, Alabama. One part was fixed in Helly fluid, imbedded in paraffin, sectioned at 6 μ, and stained routinely with hematoxylin and eosin. Representative sections were stained with thionine. One part was fixed in formalin, alcohol, and calcium acetate, imbedded and sectioned in a similar manner, and stained with toluidine blue.[6] Mast cells were counted in a given section and stained either with thionine or toluidine blue. The surface area of the section was measured with a compensating polar planimeter at 8×. The ratio of cells to surface area was then computed and recorded as cells per square millimeter of surface area of tissue.

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TABLE 1

MAST-CELL DENSITY IN SECTIONS OF CLINICALLY NORMAL AND INFLAMED GINGIVA

Author(s) and Date	Gingiva	Zone	Number of Mast Cells	Characteristics of Mast Cells	Note
Wislocki and Sognnaes, 1950	Normal, young child (1 section)	Ground substance of stroma	30–60 per high-power field ×280	Granules intensely metachromatic	Stroma of gingival tissue varies; no suggestion as to the variability in mast-cell density of various connective tissues
	Normal, monkey (1 section)	Ground substance of stroma	Exceptional numbers		
Carranza and Cabrini, 1955	Normal (14 cases)	Epithelial attachment and epithelium of gingival sulcus, with their adjacent corium	1.9 (0 to 5) (per field)		Mast cells in human gingiva are generally around blood vessels including arteries, and in the connective tissue
		Outer gingival epithelium with its adjacent corium	4.6 (2 to 10) (per field)	Elongated or with prolongation; some in contact with basal cells; some infiltrated epithelium and sent out long prolongations	In fibrous skeleton, they take on a rounded or oval shape without prolongations; farther away from the central zone condensed forms disappear, forms with prolongations appear
		An intermediate zone of corium	5 (per field)	Rounded forms with compact cytoplasmic granules	
	Acute necrotizing gingivitis (4 cases)	Neighborhood of ulcerated zone	Scant		There is a constant mobilization of mast cells from central zone toward the epithelium
		Tissue which forms the ulcer	Practically none		
	Chronic marginal gingivitis (4 cases)	Areas of cellular filtrations	Decrease		
		Rest of connective tissue	Usual proportions		
Dewar, 1955	Normal	Epithelium plus connective tissue	18.3 (S.D. 14.3) per sq. mm.		
Stella, 1955	Normal	Chorium	Numerous	Round forms surrounded by a clear, amorphous, and granule-free halo	An involutive intraepithelial cycle starts in stratum germinativum and ends at stratum corneum; granular dispersion and probable lysis with final destruction of cytoplasm and nucleus
		Epithelium	Presence constant		
	Pregnancy gingivitis		More numerous	Polymorphism strong; various stages of cytoplasmic degranulation; direct division and nuclear variability	

TABLE 1—*Continued*

Author(s) and Date	Gingiva	Zone	Number of Mast Cells	Characteristics of Mast Cells	Note
Dewar, 1958.....	Periodontitis (18 cases)	Epithelium plus connective tissue Connective tissue only	30 (S.E. 11) per sq. mm. 47 (S.D. 17) per sq. mm.		Histamine concentration 60 mu. gm. per gm. wet weight total inflamed tissue
Calonius, 1959....	Normal (13 cases)	Papillae Dorsal to 3d Molar	Entire experimental material	Slightly oval to oblong; vacuolated cells seen in all specimens	Healthy gingival papillae contained significantly more mast cells than inflamed gingival papillae
	Inflamed (11 cases)	Papillae Beneath denture	123 ± 88.9 mast cells range 20 to 400 in area of 1.16 sq. mm.	Subepithelial regions have large accumulations; other sites almost free	
	Healthy cicatricial (17 cases)	Post-extraction Beneath denture Areas of cellular infiltrations			
Dummett, Bolden and Ashhurst, 1960.....	Diphenylhydantoin sodium hyperplasia (9 cases)	Attached and free gingiva	0.47–146.00 per sq. mm. surface area	Variable size and shape; degranulation an inconstant feature	No apparent correlation between mast-cell density and (1) density of connective tissue, (2) duration of diphenylhydantoin sodium therapy, (3) degree of inflammatory cell infiltrate

RESULTS

Clinical diagnosis.—The gingivae of all nine patients were clinically hyperplastic and obscured practically the entire clinical crowns of the teeth. At biopsy, the tissues were firm, nodular, and irregular in outline (Fig. 1). The tissues varied from pink to the blue-black color of heavy melanin pigmentation.

Microscopic examination.—Sections stained with hematoxylin and eosin showed three principal features: (1) hyperplasia of the epithelium, (2) increased deposition of dense collagen, and (3) increased vascularity with variable numbers of chronic inflammatory cells (Fig. 2).

FIG. 1.—Intraoral photograph, showing clinical appearance of gingiva of patient on protracted course of diphenylhydantoin sodium.

Mast cells.—Mast cells are large connective tissue cells containing abundant cytoplasm densely laden with coarse granules. Mast cells stain metachromatically with thionine, and selectively following formalin, alcohol, calcium acetate fixation.[6] Mast cells of variable size and shape were observed with both procedures (Fig. 3). Some were oval; others were spindle-shaped. These were in the corium and predominantly near blood vessels. The nucleus was frequently obscured by the dense granules. Degranulation was an inconsistent feature (Figs. 4 and 5).

Quantitative analysis.—The actual number of mast cells counted in the nine specimens varied from 1 to 372 (Table 2). The computed mast-cell density varied from 0.47 to 146.00 mast cells per square millimeter of surface area (Table 2).

No apparent correlation could be drawn between (1) mast-cell density and duration

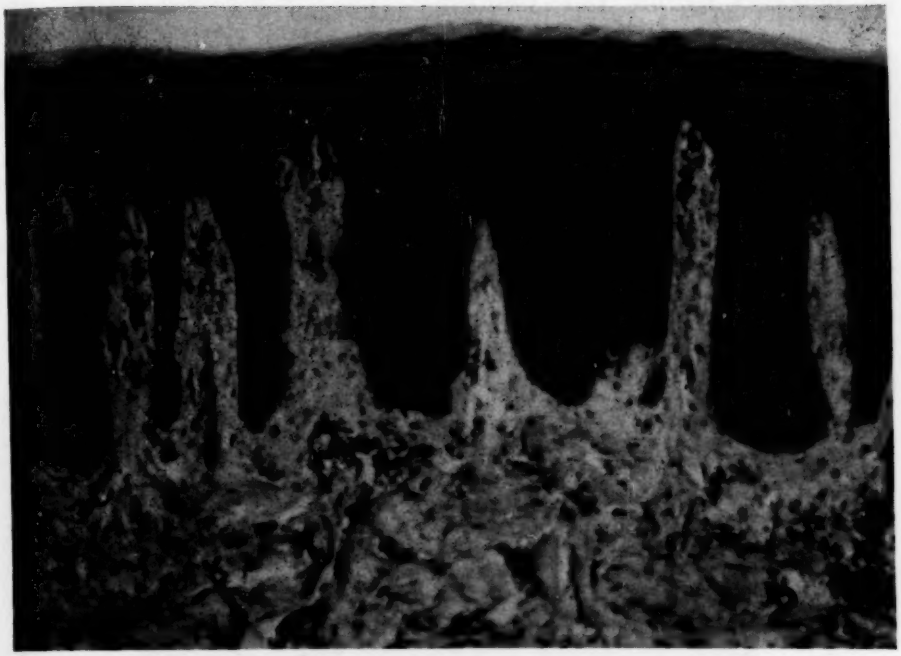

FIG. 2.—Photomicrograph, showing characteristic histologic features of section of gingiva of patient shown in Fig. 1. Hematoxylin and eosin. Mag. ×190. Reduced.

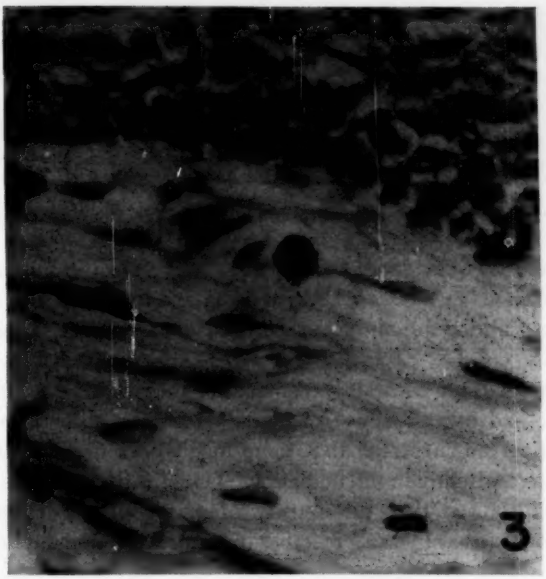

FIG. 3.—Photomicrograph, showing mast cells within the corium. Thionine. Mag. ×1,350. Reduced

of therapy, (2) mast-cell density and degree of vascularity, and (3) mast-cell density and degree of reactive response.

DISCUSSION

The exact role of the mast cell in inflammation is as yet not known but has been interpreted as a sign of healing.[4] Ehrlich[7] observed that the number of cells within the connective tissue which contained meta-chromatically stained granules was greater in the presence of chronic inflammation. Riley[8] has shown that the tissue mast cells disappear in areas of acute tissue injury and reappear when connective tissue fibrils begin to be laid down and the ground substance is shrinking. Bolden[9] demonstrated that the number of mast cells decreased in acute inflammation and increased in subacute inflammation.

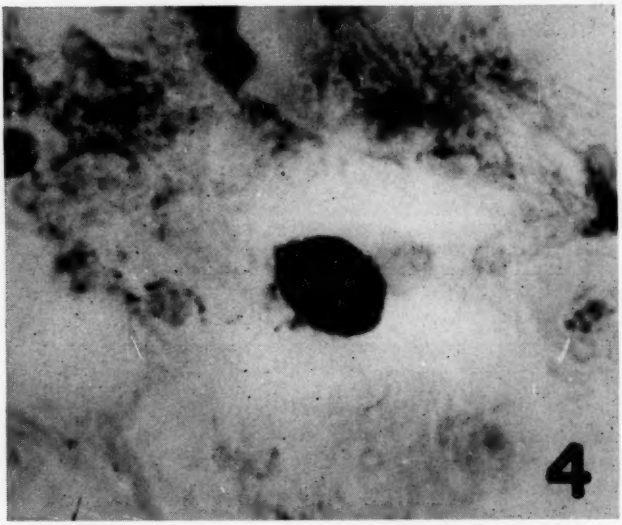

FIG. 4.—Photomicrograph of a mast cell with degranulation. Toluidine blue. Mag. ×1,900. Reduced

There is a large body of evidence that demonstrates a significant correlation between the density of mast cells in tissues with hyperemia and increased vascular permeability and between mast-cell disruption, histamine release, and edema formation in response to a given irritant.[10]

The gingivae are almost constantly responding to irritants and may show a wide range of vascular and other reactive changes.[11] Previous observations made on sections of human gingivae suggested that there might be a decrease in the number of mast cells in acute and chronic inflammation.[1] Quantitative analysis of nine samples of human gingiva showed that the density of mast cells is extremely variable in gingival tissues conditioned by, and reactive to, protracted diphenylhydantoin sodium therapy.

The production of mast cells may be influenced by radiation and rapidity of circulation[12] and may be regulated by hormonal control. An elevation of gonadotrophic

hormone may account for the increase in mast cells observed in gingivitis of pregnancy.[2] The precise nature of the response of mast cells to diphenylhydantoin sodium awaits laboratory investigation.

Mast cells may be an important source of histamine, which can be released into the tissue probably through degranulation of mast cells.[13, 14] Francis and Melville[15] have shown that the amount of extractable histamine appeared to be increased in gingivae of animals treated with diphenylhydantoin. Dewar,[4] however, was unable to demonstrate a significant correlation between the histamine content and the mast-cell

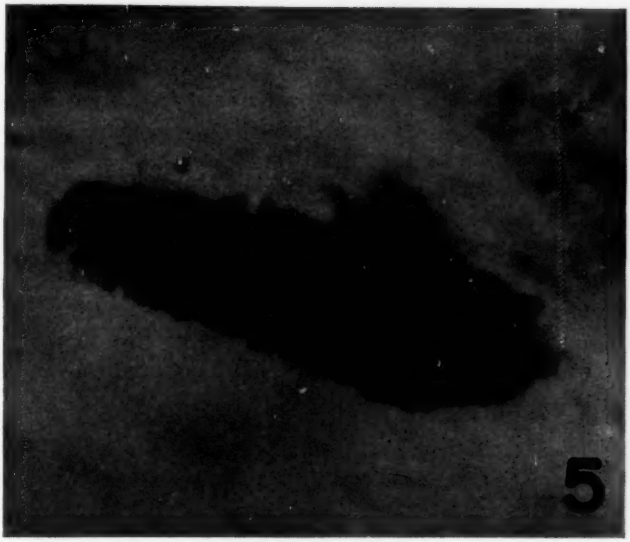

FIG. 5.—Photomicrograph of mast cell with degranulation. Thionine. Mag. ×2,475. Reduced

TABLE 2

DENSITY OF MAST CELLS IN SECTIONS OF GINGIVA FROM EPILEPTIC PATIENTS ON DIPHENYLHYDANTOIN SODIUM THERAPY

Specimen No.	Mast Cells Counted	Surface Measured	No. of Mast Cells per Sq. Mm. Surface Area	Period of Diphenylhydantoin Sodium Therapy (Years)
1.....	100	2.58	25.24	8
2.....	31	1.06	18.71	9
3.....	48	1.64	18.70	3
4.....	1	1.34	0.47	9
5.....	372	1.63	146.00	11
6.....	69	2.13	16.03	5
7.....	121	3.07	25.22	7
8.....	70	1.21	3.71	4
9.....	41	2.58	10.17	3

concentration of individual samples of inflamed gingival tissue. A similar analysis on samples of human gingival tissues reactive to diphenylhydantoin may be of significance.

The release of mast-cell granules may initiate a change in the connective tissue ground substance by changing the intercellular fluid or free tissue water into a mucinous fluid.[14] Degranulation of mast cells has been described in pregnancy gingivitis[2] and was observed in the present study. Mucinous changes, however, were not noted. They may have been replaced or obscured by the abundant dense collagen.

Marked variations in mast-cell density were noted in the sections of gingivae from controlled epileptic patients. Similar variations had been noted in sections of normal gingiva and inflamed gingiva.[1–4] This fluctuation is difficult to explain but must be physiologic, rather than purely a function of fixation.[4]

Mast cells were predominantly in the corium, although a few had migrated into the epithelium, as previously shown.[3, 4]

SUMMARY

Mast-cell density was determined in specimens of gingiva from nine patients on a protracted course of diphenylhydantoin sodium therapy. It was quite variable and ranged from 0.47 to 146.00 mast cells per square millimeter of surface area. The data on the number of mast cells in sections of normal and inflamed gingiva were summarized. Apparent variations in response of mast cells are noted.

Speculations as to the role of hormones and other reactive materials in mast-cell response are drawn. The morphology and distribution of mast cells in our sample are described.

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Radiation-induced Changes in the Epithelium of the Buccal Mucosa

*LAWRENCE P. CHASE, PATRICK D. TOTO, and MARION F. MAGALOTTI**
Loyola University School of Dentistry, Chicago, Illinois

The normal oral epithelium is subjected to ionizing radiation during the treatment of oral carcinoma by X-irradiation. Erythema, edema, inflammation, and ulceration are manifestations of the reaction of oral mucosa to therapeutic doses of X-irradiation. It was felt that sensitivity to ionizing radiation should be relatively greater in the proliferating oral epithelium than in the supporting connective tissue of the oral mucosa. Changes in the maturation of oral epithelium might occur in the absence of any clinical signs.

The maturation of oral epithelium studied by the Papanicolaou method is well established.[1] Also, it has been found accurate in detecting oral carcinoma indicated by close correlation with biopsy.[2, 3] The normal distribution of exfoliated cells, non-keratinized, partially keratinized, and completely keratinized has been established for the buccal epithelium.[4, 5]

The process of keratinization in the skin has been studied extensively, yet the mechanism has not been elucidated.[6–9] Histochemical methods offer the best method for investigating keratinization.[10, 11] There is good correlation between local irritation of the skin and keratinization.[6, 12, 13]

The interpretation of keratinization by histologic methods is largely dependent on the fixation of tissues.[14–18] Oxidation of tissues before or during fixation may alter acidophilia or basophilia as seen in stained sections. Oxidation of stratified squamous epithelium may lead to erroneous interpretation of the degree of keratinization.

Hyperkeratosis is one of the reactions observed to occur in the skin as a reaction to ionizing radiation. Not only local reactions occur, but also systemic reactions, namely, radiation sickness.[19–21] While local reactions to ionizing radiation cause changes in the chemistry and morphology of tissues, reduction in mitotic activity, oxidative effects, and vascular changes constitute the basis for such changes.[20–35]

The study of exfoliated oral epithelial cells by means of the Papanicolaou method should serve to indicate any changes in keratinization, particularly by observing any change in the distribution of non-keratinized, keratinizing, and keratinized cells.

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* Director, Radiation Center, Cook County Hospital, Chicago, Illinois.

MATERIALS AND METHODS

Thirteen human subjects, eleven men and two women, whose ages ranged from forty-one to eighty-three, all diagnosed with oral squamous-cell carcinoma, were selected for therapy by X-radiation (Table 1). None of the subjects had received any previous radiation therapy.

The clinically normal buccal mucosa was selected as the test site because of its accessibility and absence of keratinization. The number of keratinized exfoliated cells from a smear of the buccal mucosa ranged from zero to 2 per cent. In each case the radiation therapist calculated the X-ray dosage delivered to the skin and buccal mucosa (Table 2). All the subjects received the X-irradiation through a Thoreus filter #3 with HVL, 3 mm. Cu.

Two smears were taken from the buccal mucosa at the level of the occlusal plane

TABLE 1

SUBJECTS WITH ORAL CARCINOMA

No.	Sex	Age	Location of Lesion	No.	Sex	Age	Location of Lesion
100.....	M	55	Soft palate	700....	M	53	Left soft palate and tonsil
200.....	M	41	Soft palate				
300.....	M	83	Anterior two-thirds of tongue	800....	M	59	Left floor of mouth
				900....	M	60	Right hypopharynx
400.....	F	65	Left lip and buccal mucosa	1000....	M	66	Left hypopharynx
				1100....	M	51	Right floor of mouth
500.....	M	71	Right tonsil	1200....	F	57	Soft palate
600.....	M	72	Right buccal mucosa and soft palate	1300....	M	57	Left tonsil

TABLE 2

X-RAY RADIATION THERAPY DATA

Patient	Sex	Age	Total Days	Total Treatments	Total r to Mucosa	Total r to Skin	Port Size (Cm.)	Sequence of Therapy*	Groups†
100...	M	55	32	24	4216	4284	10×10	E	A
200...	M	41	29	24	4320	4366	8×10	E	A
300...	M	83	21	15	4118	4295	6× 8	E	A
400...	F	65	20	15	4650	4725	10×10	E	A
500...	M	71	10	5	1187	1206	10×12	S	A
600...	M	72	47	15	3600	3720	8×10	S	A
700...	M	53	43	16	4224	4257	10×15	S	B
800...	M	59	34	21	3718	4000	10×12	E	A
900...	M	60	40	13	2340	2423	8×10	S	B
1000...	M	66	40	11	2640	2764	7×10	S	C
1100...	M	51	21	16	3712	3872	6× 8	E	A
1200...	F	57	42	16	4130	4347	8× 8	S	C
1300...	M	57	34	21	4080	4216	8×10	E	A

* Sequence of therapy: consecutive days (E); alternate days (S).

† Groups: first group (A); second group (B); third group (C). Group A is the largest group and one in which the green cells showed a decrease in relative number and the red cells an increase at the eighteenth day. This change remained. Tan cell numbers increased throughout the therapy. Group E showed a very late decline of the green-cell numbers with a concomitant late rise in red-cell numbers. Tan-cell numbers rose. Group C showed erratic behavior. The green-cell numbers decreased at the thirteenth day and rose again in 3 or 4 days, to remain high for the remainder of therapy. A compensatory curve was shown for the red cells. Tan-cell numbers rose throughout therapy. (See also Figs. 1, 2, 3.)

with a tongue blade 24 hours before the initiation of radiation therapy and at 24-hour intervals thereafter until the completion of therapy. Both smears were placed side by side on a glass slide and immediately fixed in equal parts of 95 per cent ethyl alcohol and ether. The smears were stained by the Papanicolaou method.[12]

A differential count of non-keratinized (green), keratinizing (red), and keratinized (tan) cells was made of each of the two smears on the slide. The two counts were added, and the averages for each of the stained cells were calculated. Thus, for each subject, a differential count was recorded at each of the 24-hour intervals. For each such interval, the per cent of green, red, and tan cells for all subjects was summed and a mean percentage calculated. The variance, S^2, was calculated for each of the green, red, and tan cells by calculating the difference between the mean per cent and the per cent value of each of the respective cells of each subject. The individual differences were squared. The squared values were summed and divided by the number of subjects represented. The standard deviation was calculated by taking the square root of the variance.[36] The mean per cent, variance, and standard deviation at 1 day prior, and 8, 13, and 32 days following radiation are reported in Table 3.

TABLE 3*

DISTRIBUTION OF EXFOLIATED CELLS

DAYS OF RADIATION	NON-KERATINIZED			KERATINIZING			KERATINIZED		
	X	S	S	X	S	S	X	S	S
0....	64.23	412.13	20.30	33.10	393.87	19.84	2.54	8.66	2.94
8....	71.34	196.83	14.02	25.59	183.42	13.54	3.05	3.31	1.81
13....	43.99	837.14	28.93	49.23	677.28	26.02	6.96	3.46	1.86
32....	27.29	604.67	24.58	54.85	816.7	28.57	17.56	46.29	6.80

* Mean = X; variance = S^2; standard deviation = S.

RESULTS

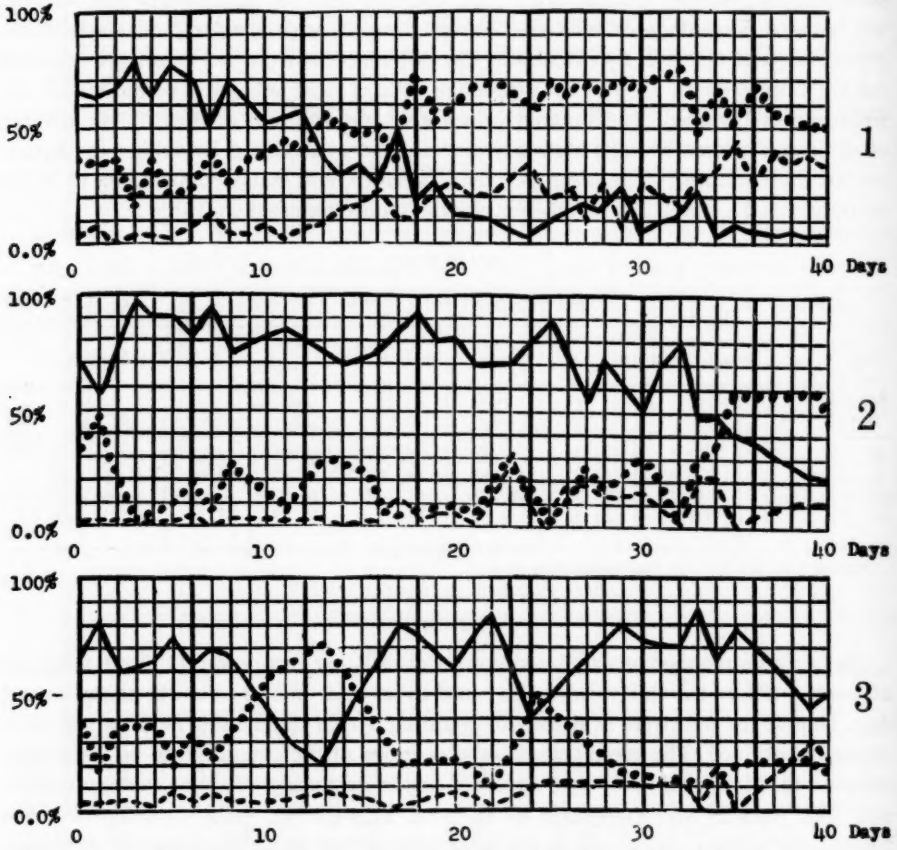
The average number of exfoliated epithelial cells per low-power (100×) field was fifty-seven before the thirty-second day and twenty-six on and after the thirty-second day. Thus fewer cells were available for counting within comparable fields as radiation therapy continued. The green cells showed portions of the cytoplasm to be irregularly stained red. More such cells were apparent in the microscopic field as therapy continued. The nucleus and cytoplasm of the red, keratinizing, cells simultaneously increased in diameter. Such cells in the later smears were twice the diameter of similar cells observed in the earlier smears.

The mean percentages of the differential counts showed a significant distribution change as radiation therapy continued; the non-keratinized cells decreased as the keratinizing and keratinized cells increased (Table 3). There were some individual differences noted as the shift in the distribution of cells occurred. Eight of the thirteen subjects showed a marked decline in non-keratinized and a rise in keratinizing cells by the eighteenth day of therapy. In two individuals an extended high count of non-keratinized cells and low-keratinizing cells continued late into therapy. Two subjects showed a rapid fall of non-keratinized cells, with concomitant rise in red cells at 13

days. Then this reversed, showing a rise in non-keratinized cells and a fall in keratinizing cells, which continued to the end of radiation therapy. These individual differences were plotted in graph form (Figs. 1–3). In all counts the number of tan cells continued to increase at a gradual rate, until, at the end of therapy, there was an eightfold increase.

DISCUSSION

The epithelium of the buccal mucosa is described as non-keratinized by histologic and cytologic studies.[2, 3, 6] There are few keratinized cells, 2.54 per cent, at the oc-



FIGS. 1–3.—Fig. 1: Group I. This group showed a decrease in green (non-keratinized) cell numbers and an increase in red (keratinizing) and tan (keratinized) cell numbers beginning on the thirteenth day. The changes in relative green-cell numbers and red-cell numbers ceased at the eighteenth day; thus the green-cell counts remained relatively low and the red counts relatively high for the remainder of therapy. The tan cells increased gradually from the thirteenth day on. Green cells = ———; red cells = ...; tan cells = - - - - -. Fig. 2: Group 2. This group showed a slightly elevated preradiation green-cell count until the thirty-second day, when this non-keratinized count decreased. The red-cell relative numbers decreased from preradiation counts until the thirty-second day, when they rose above preradiation values. The tan-cell numbers showed a gradual, but sporadic, increase throughout therapy. Fig. 3: Group 3. This group showed erratic changes in distribution of the green and red cells. At the eighth day the green-cell counts decreased, and the red-cell counts increased from preradiation values; a reversal of this occurred at the eleventh day. On the twenty-second day the green and red counts began to return to their preradiation values. Another reversal of green and red counts began on the thirty-fifth day. The tan-cell counts were expressed by a gradual rise throughout therapy.

clusal plane of the buccal mucosa, which is often subjected to trauma. Our findings cannot be interpreted to represent the entire buccal mucosa.

The reduction in the number of available cells for smears late in radiation therapy is interpreted as a diminution of mitotic activity as a result of ionizing radiation. The reduction in non-keratinized cells indicates that fewer cells are beng produced which could be available for exfoliation. Also, the non-keratinized cells remain in situ longer, as the turnover of the epithelium is suppressed. This permits the non-keratinized cells to remain in situ long enough to keratinize and eventually to increase the number of keratinized exfoliated cells. Also, if there is a reduction in the production of cells and if the epithelium is to continue to protect the underlying connective tissues, the epithelial cells present would have to remain in place longer. Under such circumstances a subnormal number of cells forced to carry on the protective function assigned to a normally greater number of cells could continue to cover the surface only by widening. This is noted in the increased diameter of the keratinizing cells. Thus a functional adaptation, expressed by an increase in the size of the keratinizing cells in the buccal mucosa, has been brought about indirectly by ionizing radiation.

The production of free radicals and peroxide within tissues as a result of ionizing radiation is known to occur.[37] The resulting reactions are essentially oxidative. There is reason to believe that oxidation of sulfhydryl groups to disulfide is operative in the mechanism of keratinization of stratified squamous epithelium. The disulfide groups of cystine are abundant in keratinized tissues.[10] Thus keratinization of parts of the cytoplasm of non-keratinized green cells, the increase in the number of keratinizing red cells, and continued increase in keratinized tan cells may be attributed to the oxidative effects of ionizing radiation.

Injury to blood vessels by ionizing radiation is a well-established fact.[28–30] Obliteration of blood vessels disturbs metabolic activity between the endothelium, interstitial space, and the epithelial cell wall. Such effects may reduce mitotic activity and cell production.

Figures 1–3 suggest the cellucidal effectiveness of the radiation therapy. Friedman and Rosh have illustrated such curves indicative of periods of cellucidal activity and healing. Figure 1 shows the response of an average individual to radiation therapy as expressed by the reduction of non-keratinized cells in his buccal mucosa. Figure 2 suggests a hyposensitive reaction as the green cells, non-keratinized, continue at a normal rate despite radiation therapy. Figure 3, however, suggests neither hypersensitive nor hyposensitive reaction. However, the number of individuals does not lend itself to a thorough analysis of radiation sensitivity but does suggest such activity.

SUMMARY

Smears of the normal buccal mucosa from thirteen subjects with oral carcinoma treated by X-radiation were taken before therapy and at 24-hour intervals during the course of therapy. Papanicolaou's method for staining keratinized and non-keratinized cells was used, and a differential count was done. A shift in the distribution of cells from non-keratinized to keratinized occurred as a result of radiation therapy. There was a decrease in the number of exfoliated cells in the 100× microscopic field as therapy progressed. Also, the size of the keratinizing cell widened to twice the diameter late in therapy, as compared with similar cells taken earlier in therapy.

The mitosis-inhibition effects of ionizing radiation on the normal oral epithelium

of the buccal mucosa caused a reduction in the number of cells. A reduced number of cells called upon to function as the lining of the oral mucosa remain *in situ* longer, permitting the normally non-keratinized cells to keratinize and increasing the number of keratinized exfoliated cells. The widening of keratinizing cells is a functional adaptation of subnormal epithelium to carry on the function of lining the oral mucosa and is an indirect effect of ionizing radiation. The increase in keratinization of normally non-keratinized cells is a function of oxidative effects within the cytoplasm of the epithelial cells. It is felt that sulfhydryl groups are converted to disulfides as in cystine, as a result of oxidation, thereby accelerating the keratinization process. The rate at which non-keratinized cells decrease during radiation therapy varies in individuals and suggests variation in cellucidal effect of ionizing radiation.

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Compressive Properties of Enamel, Dental Cements, and Gold

R. G. CRAIG, F. A. PEYTON, and D. W. JOHNSON
University of Michigan, School of Dentistry, Ann Arbor, Michigan

The compressive properties of human enamel and dentin have been reported by Stanford, Paffenbarger, Kumpula, and Sweeney.[1] The elastic modulus of occlusal, side, and cusp enamel was reported to be 1.8, 6.0, and 8.2 $\times$ 10^6 psi, respectively. The corresponding values for the proportional limit were 16,800, 21,000, and 34,200 psi and, for the compressive strength, 19,400, 28,300, and 40,200 psi.

An improved procedure for preparing compressive specimens of hard tooth tissues and some restorative materials was published by Stanford, Weigel, Paffenbarger, and Sweeney.[2] The compressive properties of enamel were within the experimental error of the earlier values, and additional values relating compressive properties to environment of development and orientation were reported. In addition, the compressive properties of plastics, amalgam, silicate cement, zinc phosphate cements, and dental golds were listed.

Tyldesley[3] determined the mechanical properties of enamel by using a transverse type of loading system. The elastic modulus of enamel was reported to be 19 $\times$ 10^6 psi in bending. The proportional limit and compressive strength were found to coincide at an average value of 11,000 psi.

The published values for the compressive strength of enamel appear low when compared with the values listed for human dentin. Craig and Peyton[4] reported an average compressive strength for dentin of 43,100 psi; Stanford *et al.*[1] gave a value of 50,400 psi; and Tyldesley[3] published a value of 38,800 for the breaking stress. The highest average compressive-strength value of 40,200 psi reported for cusp enamel is, in general, lower than those reported for dentin.[1, 2] These results do not appear reasonable when the hardness and general working characteristics of enamel and dentin are compared.

The principal purpose of this investigation, therefore, was to re-evaluate the compressive properties of proportional limit, compressive strength, and elastic modulus of human enamel, using improved procedures for sample preparation.

In addition, the dental literature included little information concerning the compressive properties of restorative materials measured on specimens approaching the size normally used in dentistry.[2] Investigations of the effect of sample size on the compressive properties of amalgam indicated that higher values were obtained with smaller specimens. The second object of this study, therefore, was to determine the compres-

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sive properties of small specimens of silicate cement, zinc phosphate cement, and dental gold and to compare these values with those obtained on large test specimens.

MATERIALS AND METHODS

Enamel.—Freshly extracted human teeth, generally mandibular first molars, were used as the source of enamel because the enamel is reasonably thick on the buccal portion of these teeth. Since the method of sample preparation is of prime importance in obtaining accurate compressive properties, a detailed description of the procedure will be given.

The teeth were mounted in a cup containing impression compound such that the cusp or side of the tooth selected for sampling was parallel to the proposed axis of the specimen. The cup was designed so that it could be mounted on the tail stock of a jeweler's lathe. A hollow, diamond core was placed in the head stock of the lathe and a cylindrical blank was cut out of the tooth, using water as a coolant. These blanks were approximately 0.060 inch in diameter and consisted of enamel on one end and dentin on the other. The core drill cut into the surface of the tooth at a right angle, and therefore the general direction of the enamel rods was parallel to the long axis of the cylinder.

The enamel end of the specimen was placed in the chuck of the jeweler's lathe, and the dentin end was machined to 3/64 inch (0.0468 inch), using a tungsten carbide tool. The dentin end was then placed in a 3/64-inch collet and the enamel end machined to 1/32-inch (0.0312 inch) in diameter. It was necessary to avoid the use of a center tip on the enamel end because the pressure of the tip caused splitting of the specimens.

After the enamel end had been machined to 1/32 inch diameter, the dentin portion was cut off. The enamel specimen was placed in a 1/32-inch collet and the ends ground off flat and parallel with an India stone and a lathe grinding attachment. The grinding operation was arranged so that it always took place from the edge toward the center of the sample, thus avoiding specimens with chipped edges. All the previous operations were carried out with water as the coolant.

The specimens were examined microscopically for any flaws, and, if none were found, the samples were stored in distilled water until the determination of the compressive properties.

Attempts to prepare enamel specimens with the rod direction perpendicular to the long axis of the cylinder have so far met with failure. Since in normal mastication the compressive forces are generally applied in the direction of the enamel rods or on the ends of the rods, the specimens prepared should yield information related to clinical experience.

Silicate and zinc phosphate cements.—A tapered, single-unit, stainless-steel mold was used to prepare blanks for silicate and zinc phosphate cement specimens. The mold yielded specimens that were about ⅛ inch in diameter and 11/16 inch long. Cylinders were prepared from these blanks in a manner similar to that described for enamel. The finished silicate cement specimens were ⅛ inch (0.0125 inch) in diameter and varied from 5/16 to ⅜ inch long; filling porcelain* was used with a powder/liquid ratio of 1.43 gm/0.4 cc.

* S. S. White Co., Chicago, Ill.

The zinc phosphate samples were more difficult to prepare than the silicate cement samples, and therefore the diameter was increased to 5/16 inch. The specimen length again was approximately 3/8 inch. The cement* was used in two consistencies. Inlay-consistency mixes were prepared with 1.1 gm. of powder for 0.5 cc. of cement liquid, and the base-consistency mixes contained 1.85 gm. of powder for 0.5 cc. of cement liquid.

Gold.—Castings were prepared from partial denture gold,† using the water-added casting procedure. The gold was quenched when the sprue button ceased to show a red glow. Gold cylinders, 0.080 inch in diameter and 3/4 inch long, were cast. Specimens were machined from the cylindrical blank with the jeweler's lathe and a tungsten-carbide tool. The dimensions of the specimens were 0.078 inch in diameter and 0.28 inch in length. It was not possible to use the grinding procedure in preparing the ends of the cylinders because of smearing of the gold. The ends were machined with the tungsten-carbide cutting tool.

The partial denture gold specimens were subjected to the following hardening heat-treatment procedure. The specimens were placed in a 50–50 $NaNO_3$-KNO_3 bath for 15 minutes at a temperature of 350° C.

Compression testing.—The proportional limit, compressive strength, and elastic modulus of the various samples were determined by the procedure described by Craig and Peyton[4] in a study of human dentin. The specimens were placed between two steel plungers, the deformation was obtained from optical strain gauges, and the load indicated by a Reihle Testing Machine. Corrections were applied to the measured strain for the deformation resulting from the shortening of the steel plungers and foundation effects as previously described.[4]

The stress-strain curves for enamel were obtained, using a discontinuous loading rate. The load was increased in increments and the strain measured at these intervals. This procedure was possible, since no flow of enamel was observed, as had been the case with human dentin.

The deformation rate used in the determination of the compressive properties of silicate cements was 0.015 inch/minute. The deformation rate for the zinc phosphate cements and gold specimens was 0.01 inch/minute.

RESULTS

Enamel.—The compressive properties of human cusp enamel are listed in Table 1 and those for side enamel are reported in Table 2. In each case, the general direction of the enamel rods was parallel to the long axis of the cylinder. Also listed in the tables are the length/diameter ratios of the various samples. The majority of the length-to-diameter ratios for cusp enamel was greater than 2, since the enamel in the cusp portion of a tooth is reasonably thick. The side enamel specimens, however, all had ratios less than 2 because of the thinner enamel layer on the side of a tooth. It was possible, however, to obtain ten specimens having a length-to-diameter ratio greater than 1.4; the remaining seven specimens had ratios ranging from 1.06 to 1.36.

The proportional limit of cusp enamel varied from 30,600 pounds/square inch (psi) to 64,800 psi, and an average value of 51,200 psi was obtained for specimens having a

* L. D. Caulk Co., Milford, Delaware.

† J. F. Jelenko & Co., Inc., New York, and the J. M. Ney Co., Hartford, Conn.

TABLE 1

COMPRESSIVE PROPERTIES OF HUMAN ENAMEL

SAMPLE	CUSP ENAMEL			LENGTH/ DIAMETER
	Proportional Limit (Psi)	Ultimate Compressive Strength (Psi)	Elastic Modulus (Psi$\times 10^{-6}$)	
1	53,900	55,200	12.3	3.00
2	40,900	44,200	11.1	2.63
3	48,800	59,000	12.5	2.37
4	62,200	66,700	13.2	2.32
5	30,600	33,200	9.6	2.14
6	57,400	63,800	13.6	2.08
7	64,800	74,400	12.7	2.01
8	.....	48,800	12.8	2.00
9	.....	67,100	10.9	1.88
10	33,400	39,000	9.1	1.85
11	39,500	43,400	11.3	1.68
12	41,200	50,700	13.9	1.56
Av.*	51,200	55,700	12.2×10^{6} $\pm 0.9\times10^{6}$	.....

* Includes samples with l/d equal to, or greater than, 2.

TABLE 2

COMPRESSIVE PROPERTIES OF HUMAN ENAMEL

SAMPLE	SIDE ENAMEL			LENGTH/ DIAMETER
	Proportional Limit (Psi)	Ultimate Compressive Strength (Psi)	Elastic Modulus (Psi$\times 10^{-6}$)	
1	31,300	36,300	11.7	1.97
2	60,300	64,200	10.6	1.74
3	49,700	59,900	12.2	1.64
4	44,800	46,600	11.8	1.64
5	48,100	49,400	10.3	1.58
6	48,800	51,300	10.8	1.55
7	57,000	60,900	12.8	1.54
8	49,700	54,100	10.9	1.41
9	37,000	53,700	11.5	1.40
10	60,200	62,800	10.7	1.40
11	37,500	41,900	10.4	1.36
12	44,600	45,300	12.4	1.31
13	37,800	39,100	10.3	1.31
14	65,800	88,200	12.1	1.25
15	62,400	81,900	10.7	1.23
16	51,000	56,800	10.0	1.10
17	62,800	78,500	12.6	1.06
Av.*	48,700	53,900	11.3×10^{6} $\pm 0.7\times10^{6}$	.....

* Includes only samples with l/d equal to, or greater than, 1.4.

length/diameter (l/d) ratio equal to, or greater than, 2. The values obtained on specimens with ratios from 1.56 to 1.88 were, however, not significantly different from those with l/d ratios greater than 2.

The ultimate compressive strength for samples with l/d greater than 2 ranged from 33,200 to 74,400 psi, and an average figure of 55,700 psi was calculated. From these data it may be seen that a wide variation in strength was obtained, possibly because of actual variations in the specimens or imperfections in the samples. There is, therefore, some question as to what an average value for the proportional limit or compressive strength represents, although it does serve as a general indication of these values for enamel. Of particular interest is the relatively small difference between the proportional limit and compressive strength for any single specimen. This means that human enamel does not become permanently deformed until shortly before fracture of the specimen.

The elastic-modulus values for cusp enamel ranged from 9.1×10^6 to 13.9×10^6 psi. The average elastic modulus was 12.2×10^6 psi, and the average deviation from the mean was calculated to be $\pm 0.9 \times 10^6$ psi. When the samples having l/d ratios less than 2 were included in the average, the elastic modulus was lowered slightly to 11.9×10^6 psi.

The compressive properties of side enamel, reported in Table 2, are within the experimental error of those obtained for cusp enamel. The average values for the proportional limit, compressive strength, and elastic modulus were 48,700, 53,900, and 11.3×10^6 psi, respectively. Specimens having l/d ratios of less than 1.4 were not included in these averages. The proportional limit and compressive-strength values obtained with specimens having l/d ratios of less than 1.3 were considerably higher than those having larger l/d ratios.[5] This increase is probably due to the samples approaching a disk, where the apices of the cone-shaped stress patterns are closer together than in longer specimens. Since the stress in the cone-shaped volume is less than in the remainder of the specimen and since in short samples the cone volume is a larger portion of the total specimen volume, short samples are able to withstand higher stress than long specimens.

The elastic moduli of the short specimens do not alter the average value when they are included. The observation that the elastic modulus is less affected by the length-to-diameter ratio than are the proportional limit and compressive strength is a typical finding in compression testing.

Silicate and zinc phosphate cements.—The compressive properties of silicate cements, 24 hours and 7 days after preparation, are given in Table 3. The proportional limit, ultimate compressive strength, and elastic modulus of the 24-hour specimens averaged 19,600, 25,600, and $3{:}25 \times 10^6$ psi, respectively. The corresponding values for the 7-day specimens were 24,300, 32,100, and 3.58×10^6 psi. Considering the errors involved in the measurements, these data indicate a small but definite increase in the compressive properties of the 7-day specimens. As in the case of enamel and other brittle materials, the proportional limit is nearly as high as the compressive strength. This indicates that little permanent deformation of the material is possible before rupture occurs. The average 7-day compressive strength of 32,100 psi was higher than the value of 28,500 reported by the manufacturer. Also it should be noted

that the 24-hour strength of 25,600 psi exceeds the A.D.A. Specification No. 9 requirement of 23,000 psi.

The zinc phosphate cement had slightly lower compressive properties than the silicate cement. Since the zinc phosphate cements are used as inlay cements and as base cements, the compressive properties of mixes of both consistencies were investigated and are reported in Table 4. The values listed for the inlay consistency mixes after 1, 7, and 28 days showed a gradual improvement in compressive properties from the time of mixing. The proportional limit, compressive strength, and elastic modulus increased from 8,600, 14,400, and 1.64×10^6 psi, respectively, after 1 day to 13,900, 21,700, and 2.36×10^6 psi, respectively, after 28 days.

TABLE 3

COMPRESSIVE PROPERTIES OF SILICATE CEMENTS*

Sample	Age (Days)	Proportional Limit (Psi)	Ultimate Compressive Strength (Psi)	Elastic Modulus (Psi$\times 10^{-6}$)
1	1	.....	25,300	3.13
2	1	18,300	25,400	3.57
3	1	17,900	26,000	3.17
4	1	20,400	25,400	3.34
5	1	21,200	25,800	3.03
Av.	.....	19,600 ±1,400	25,600 ±300	3.25×10^6 $\pm0.17\times10^6$
6	7	26,900	29,900	3.76
7	7	.....	32,400	3.66
8	7	23,200	27,400	3.41
9	7	26,100	37,800	3.45
10	7	20,900	32,700	3.61
Av.	.....	24,300 ±2,200	32,100† ±2,700	3.58×10^6 $\pm0.12\times10^6$

* S. S. White Improved Filling Porcelain, 1.43 gm. cement powder for 0.4 cc. of cement liquid.
† The manufacturer's value was 28,500 psi.

The compressive properties of the zinc phosphate cement base-consistency mixes, measured 1 and 7 days after preparation, indicated only a slight increase in strength over this period. The base-consistency mixes had higher strengths than the inlay mixes, as shown by the average proportional limit, compressive strength, and elastic modulus values of 13,900, 26,400, and 3.40×10^6 psi, respectively.

Gold.—A type I and a type IV gold were used in a limited investigation of the compressive properties of dental golds, and the results are reported in Table 5. The average proportional limit of the type I gold was 17,700 psi, and the elastic modulus was 11.2×10^6 psi. No value could be obtained for the compressive strength, since the large permanent deformation prevented any true rupture of the specimens in compression. The proportional limit in compression of 17,700 psi was considerably higher than the value of 12,000 psi measured in tension by the manufacturer. The value of 11.2×10^6 psi for the elastic modulus in compression, however, was in excellent agreement with the tension value of 11.0×10^6 psi.

The partial denture gold specimens were tested in the hardened condition. An average value of 74,600 psi was established for the proportional limit, and a figure of 14.4×10^6 psi was found for the elastic modulus. The values obtained on individual specimens were in close agreement with the average values. The proportional limit in compression of 74,600 psi was again higher than the manufacturer's value of 72,000 psi obtained in tension. No figure was listed for the elastic modulus in tension. As in the case of the type I gold, it was impossible to obtain a true ultimate compressive strength for the partial denture gold.

DISCUSSION

The compressive properties of cusp and side enamel have been shown to be essentially equal. The average values for the proportional limit, compressive strength, and elastic modulus of 51,200, 55,700, and 12.2×10^6 psi, respectively, are considerably

TABLE 4

COMPRESSIVE PROPERTIES OF ZINC PHOSPHATE CEMENT*

Sample	Age (Days)	Mix Type	Proportional Limit (Psi)	Ultimate Compressive Strength (Psi)	Elastic Modulus (Psi$\times 10^{-6}$)
1	1	Inlay†	8,600	15,100	1.56
2	1	Inlay	8,100	14,200	1.55
3	1	Inlay	8,700	14,100	1.63
4	1	Inlay	8,800	13,300	1.66
5	1	Inlay	8,900	15,200	1.78
Av.			8,600	14,400	1.64×10^6
6	7	Inlay	11,500	18,500	2.23
7	7	Inlay	13,500	17,200	1.96
8	7	Inlay	14,400	17,500	1.94
Av.			13,100	17,700	2.04×10^6
9	28	Inlay	13,100	23,800	2.56
10	28	Inlay	14,100	24,200	2.59
11	28	Inlay	14,300	21,400	2.16
12	28	Inlay	13,900	20,500	2.36
13	28	Inlay	13,900	18,500	2.10
Av.			13,900	21,700	2.36×10^6
14	1	Base‡	13,900	25,900	2.99
15	1	Base	14,100	25,600	3.24
16	1	Base	13,700	24,700	3.20
17	1	Base	14,200	24,700	3.05
Av.			14,000	25,200	3.12×10^6
18	7	Base	13,600	25,200	3.56
19	7	Base	13,900	26,300	3.16
20	7	Base	14,300	27,800	3.44
21	7	Base	13,700	26,200	3.43
Av.			13,900	26,400	3.40×10^6

* Tenacin Cement, L. D. Caulk Co., Milford, Delaware.
† 1.1 gm powder/0.5 cc cement liquid.
‡ 1.85 gm powder/0.5 cc cement liquid.

greater than the corresponding figures of 24,200, 43,100 and 2.65×10^6 psi previously reported for human dentin.[4]

The proportional limit of cusp and side enamel was reported by Stanford *et al.*[1, 2] to be 32,500–34,200 and 21,000–27,000 psi, respectively. The compressive strengths for comparable positions of enamel were 37,800–40,200 and 28,200–34,600 psi, respectively. These values are from 28 to 59 per cent lower than those found in the present research. Since the length-to-diameter ratios of Stanford's specimens varied from 1.3 to 2.1, the sample size should not have markedly affected the results. Lower ratios should give, in any case, higher, rather than lower, proportional limit and compressive-strength values.

The elastic modulus of cusp and side enamel was reported by Stanford *et al.*[1] to be 8.2×10^6 and 6.0×10^6 psi, respectively. More recent values by Stanford *et al.*[2] list

TABLE 5

COMPRESSIVE PROPERTIES OF DENTAL GOLD

Sample	Type	Proportional Limit (Psi)	Elastic Modulus (Psi$\times 10^{-6}$)
1.....	I*	20,800	11.5
2.....	I	16,300	11.1
3.....	I	16,900	10.5
4.....	I	17,000	11.8
Av.....	.....	17,700	11.2×10^6
5.....	IV†	74,400	14.5
6.....	IV	76,300	14.8
7.....	IV	73,700	14.0
8.....	IV	74,000	14.3
Av.....	.....	74,600	14.4×10^6

* Jelenko Special Inlay; yield strength given as 12,000 psi, and elastic modulus as 11.0×10^6 psi, J. F. Jelenko & Co., Inc., N.Y.

† Ney G-3 ORO, hard condition; proportional limit listed as 72,000 psi, the J. M. Ney Co., Hartford, Conn.

these values as $6.7 \pm 0.7 \times 10^6$ and $4.7 \pm 0.6 \times 10^6$ psi, respectively. These values are substantially lower than the figures of 12.2×10^6 and 11.3×10^6 psi found in the present study.

The lower values for these properties possibly indicate imperfections in the enamel specimens, since any flaws would result in lower compressive properties. The lack of agreement between the results of the studies also may be due to differences in the method of preparing the very small specimens or to variations in the surface finish of the samples.

The higher compressive-strength properties of enamel compared with dentin, observed in the present investigation, correlate with the known properties of these materials. Higher compressive properties for dentin compared with enamel, found by Stanford *et al.*,[1] are difficult to reconcile with the general differences between these materials.

The elastic modulus and strength in bending of 19×10^6 and 11,000 psi, respective-

ly, reported by Tyldesley,[3] are in disagreement with the current values for the compressive properties of enamel. It is questionable whether the results observed in bending, for a non-isotropic material such as enamel, can be compared with those in compression, particularly since the bending specimens were a composite of enamel and dentin.

The proportional limit, compressive strength, and elastic modulus of silicate cement (24,300, 32,100, and 3.58×10^6 psi) were of the same order of magnitude as the corresponding values for dentin (24,200, 43,100, and 2.65×10^6 psi). The elastic modulus of silicate cements in compression is also in good agreement with the modulus in tension of 3.1×10^6 psi, reported by Bowen and Rodriguez.[6] It is also interesting to note the good agreement between the elastic modulus of 2.8×10^6 psi for dentin in tension[6] and the value of 2.65×10^6 psi for the modulus in compression.[4]

The compressive properties of the zinc phosphate cement specimens prepared from inlay-consistency mixes were lower than those for silicate cements and dentin. When a base-consistency mix was tested, the compressive properties were within reasonable agreement with those obtained on silicate cements. The greatest difference between the zinc phosphate cements and silicate cements was the markedly lower proportional limit of the zinc phosphate cements. The compressive properties of silicate and zinc phosphate cements reported by Stanford *et al.*[2] are in reasonable agreement with the 1-day values obtained in the present study. The elastic modulus of the 28-day inlay-consistency zinc phosphate cement samples was essentially the same as the values for human dentin. The base-consistency zinc phosphate cement and the silicate cement samples had elastic modulus values between 3.40 and 3.58×10^6 psi compared with an average value of 2.65×10^6 for human dentin.

The compressive properties of the dental gold specimens showed that these materials had elastic moduli similar to human enamel. The proportional limit of the type I gold, however, was 35 per cent less than that for enamel, and the value for the type IV gold was 50 per cent greater. Thus considerable difference exists between gold and enamel in respect to the stress at which permanent deformation will result. There is also a great difference in the amount of permanent deformation tolerated before rupture.

In general, it was observed that the proportional limits and compressive strengths of the small specimens used in this study were higher than those reported for larger specimens. This relationship between strength and specimen size has been previously observed by Taylor, Sweeney, Mahler, and Dinger[7] in a study of the factors influencing the crushing strength of dental amalgam. This relationship should be kept in mind when comparing literature data.

SUMMARY

The compressive properties of enamel, silicate cement, zinc phosphate cement, and dental gold were determined.

Cusp and side enamel were shown to have similar compressive properties, which were substantially higher than those for dentin.

Silicate and zinc phosphate cements were found to have compressive properties of the same order of magnitude as that determined for human dentin. The age of the

specimens and the powder-to-liquid ratio affected the compressive properties of the cements.

Type I gold had a proportional limit substantially lower than tooth structure, while type IV gold, in the hardened condition, had a proportional limit considerably higher than enamel or dentin. The elastic moduli of type I or IV gold, however, were comparable to values obtained for human enamel.

The compressive properties obtained for the various materials showed that higher values were obtained for the proportional limit and compressive strength when small, rather than large, specimens were utilized.

The proportional limit and compressive-strength values for enamel were found to be higher than usual when the length-to-diameter ratios of the cylindrical specimens were less than 1.3.

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Caries Resistance in Golden Hamsters

*P. J. HOLLOWAY**

Medical Research Council, London, England

After Arnold[1] had described the presence of carious lesions in the molar teeth of golden hamsters and this finding had been confirmed from independent sources,[2–5] it appeared that a very suitable caries-susceptible species had been discovered. However, an attempt by the author to induce dental caries in a strain of hamsters kept at the National Institute for Medical Research (NIMR) failed. Twenty-four weanling hamsters were fed two high-sucrose diets (Table 1) for more than 6 months, but only two carious lesions were seen in their molar teeth (Table 2, Expt. 1). This paper describes investigations carried out in an attempt to study this apparent resistance further.

EXPERIMENTAL METHODS

The hamsters were reared at NIMR from 1953 to 1957. The animals in the breeding colony were kept in metal cages with sawdust on the floors and wood-wool for bedding. They were provided with a pelleted, laboratory stock ration† (fluorine content 15 mg/kg) supplemented with green vegetables (average fluorine content 0.5 mg/kg) and tap water (fluorine content 0.2 p.p.m.). Litters were weaned at 21 days.

Similar experimental conditions were maintained throughout the study, except where otherwise stated. At weaning, litters were transferred to screen-bottomed cages with no bedding. Usually three or four male or female hamsters were kept together.

Experimental diets were fed ad libitum in aluminium trays covered with galvanized-wire mesh to reduce wastage. Fresh diet was provided every other day, and any residue discarded. Demineralized water was given from glass drinking bottles. The compositions of the diets are listed in Table 1, and the experimental procedures summarized in Table 2. Room temperature was maintained at about 27° C. (80° F.), and, in all the experiments except one, only hamsters from the NIMR colony were used.

Five separate experiments were conducted in an attempt to find the cause of the inactivity. The first of these (Expt. 2, Table 2), was a comparison between the caries activity of the NIMR and two other strains. A few stock animals were obtained from two independent sources—the Chester Beatty Cancer Research Institute, London, and the Agricultural Research Station at Compton. Small colonies were reared separately

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† Diet 41b—ground wheat, 47 per cent; ground oats, 40 per cent; white fish meal, 8 per cent; drodisol yeast, 1 per cent; dried skimmed milk, 3 per cent; NaCl, 1 per cent; plus vitamins A, D, B_1, B_2, B_{12}, E, and K, choline, pantothenic and nicotinic acids. Manufacturers: E. Dixon & Co., Ware, Hertfordshire, England.

TABLE 1

Composition of Diets (Gm.)

Ingredients	Diets			
	No. 1	No. 2	No. 4*	No. 6*
Granulated sugar	47	70	48	50
Roller processed whole-milk powder	30	.....	25	25
Crude casein	10	18	10	10
Arachis oil	5†	2	5‡	5§
Salt mixture‖	4	3	4	.....
Calcium-deficient salt mixture#	.....	.....	.....	2.75
Cellulose powder	3	.....	3	3
Dried yeast	.....	2	5	5
Vitamin mixture 1‖	1	.....	.....	.....
Vitamin mixture 2‖	0.2	.....	.....	.....
Dried cabbage	.....	5	.....	.....

* To every 100 gm. diet was added 20 mg. ascorbic acid.

† Oil contained 1,000 I.U. vitamin A and 100 I.U. vitamin D.

‡ Oil contained 1,000 I.U. vitamin A, 100 I.U. vitamin D, and 2.5 mg. *dl*-α-tocopheryl acetate.

§ Oil contained 1,000 I.U. vitamin A and 2.5 mg. *dl*-α-tocopheryl acetate.

‖ As for diet 58[8].

Calcium-deficient salt mixture: NaCl, 43.3; $MgSO_4 \cdot H_2O$, 76.5; $NaH_2PO_4 \cdot 2H_2O$, 133.0; K_2HPO_4, 366.6; $CuSO_4 \cdot 5H_2O$, 1.25; $FeC_6H_5O_7 \cdot 5H_2O$, 29.5; KI, 0.125; $MnSO_4 \cdot H_2O$, 3.79.

TABLE 2

Details of Experiments

Expt.	No. of Animals	Days Duration	Strain of Hamster	Diets		Total No. of Cavities
				During Reproduction	After Weaning	
1.....	10 14	100–200 250	NIMR	Stock	No. 1 No. 2	0 2
2.....	16 14 12	270	NIMR Compton CB	Stock	No. 4	1 0 1
3.....	24 5	270 172–242	NIMR	Stock No. 6	No. 6	0 1
4.....	45 20	210–300 250–330	NIMR	Stock to birth, No. 4 During lactation, No. 4	No. 4	8 4
5.....	24	250	NIMR	Stock	No. 4	0
6.....	20 4	250	NIMR	Stock	No. 4	98 4

under conditions similar to those of the NIMR strain, to insure a similar dietary history during the reproductive cycle. Weanling animals from all three strains were fed diet No. 4 and were killed 270 days later.

Experiment 3 was an attempt to increase the caries activity of the NIMR strain by reducing the nutritional value of the developmental diet. Stock animals were selected at weaning and fed diet No. 6, a modification of diet No. 4 in which the calcium and vitamin D content had been reduced by removing the calcium salts from the mineral mixture and the calciferol from the vitaminized oil. When 3 months old, they were mated, but only two litters were born, and, of these, only two pups survived until weaning. These were maintained on diet No. 6 until they died, 172 days later. The original animals were killed 270 days after they had been weaned.

Because of the poor reproduction, adult animals from the stock colony were given diet No. 6 and bred. Again two litters were produced, but only three animals survived. These were fed diet No. 6 for over 200 days.

In the fourth experiment the effect on caries activity of feeding a synthetic diet at two different stages of development was tested. Pregnant hamsters were transferred from stock ration to diet No. 4 as soon as their litters were born, while others were fed diet No. 4 throughout the whole of their pregnancy. The offspring were killed at varying ages between 210 and 330 days.

The fifth experiment involved alterations in the regimen, in an attempt to influence caries activity. The experimental cages were fitted with solid metal floors to prevent the excreta from falling through out of reach of the hamsters, and, in addition, half the group had the experimental diet (No. 4) added to the residue as required, instead of the usual practice of discarding the food not eaten every other day. All the animals were killed after 250 days on diet.

The final test was carried out to decide the effect of sialoadenectomy on caries activity. The animals were fed diet No. 4 and were operated on between 31 and 34 days of age. Both pairs of submaxillary and sublingual glands were removed and the parotid ducts ligated. They were killed 250 days after weaning (Expt. 6, Table 2).

When the animals were killed, the jaws were separated, trimmed of soft tissue, and fixed in 10 per cent formol-saline. They were subsequently dried and the teeth examined for carious lesions with a dissecting microscope (mag. $\times 20$).

RESULTS

Table 2 shows that, in all the experiments except the last, a very low caries incidence was maintained. Sections through the incisor teeth of the animals fed diet No. 6 in the third experiment showed these to be poorly calcified, and, on macroscopic examination, there was a considerable amount of food debris in the fossae and sulci of the molar teeth. The poor reproduction in this experiment, which was probably a consequence of the inadequate nutrition, resulted in a lack of conclusive data about the developmental effect of the diet.

The altered regimen in the fifth experiment meant that the animals were less clean than those of previous tests, and coprophagy was more common. Those whose food was not regularly changed showed signs of intermittent gastrointestinal infections.

As it was possible that the relatively high fluorine content of the stock ration might have influenced the caries resistance of these animals, fluorine estimations on the molar

teeth of some of the groups were made. Animals from the stock colony had 22.9 mg fluorine/100 gm dry weight of crown substance; animals weaned from stock diet and transferred to diet No. 4 had 11.7 mg/100 gm; and animals bred and kept on diet No. 4 had 2.9 mg/100 gm. Despite the considerable reduction in the fluorine content of the teeth of the last group, the caries inactivity of these animals was still very obvious (Table 2, Expt. 4).

In contrast to all the previous measures, sialoadenectomy had a very marked effect on the teeth. In the twenty animals examined, 98 lesions were seen, whereas, in four unoperated littermates, only 4 carious teeth were present. The lesions seen in the operated animals were of three types: 44 per cent appeared to commence around the amelo-cement junction; 40 per cent occurred on the occlusal surfaces but seemed similar to attrition areas; and the remainder looked similar to the few lesions seen in the sulci of the unoperated animals.

DISCUSSION

Most studies in experimental dental caries involve the use of caries-susceptible animals. However, there are reports of caries resistance in laboratory species, and some give details of attempts to discover the cause of the resistance. In particular, this problem has been investigated in rats both at Michigan, with the Hunt-Hoppert caries-resistant and caries-susceptible strains, and at Harvard, with the Harvard caries-susceptible and caries-resistant colonies. Despite this work, the factors controlling the resistance to caries are not fully understood.

In the present study, care was taken to reproduce experimental conditions and diets as similar as possible to those used by other investigators who have reported the production of caries in experimental animals.[5] In addition, test periods were used which were in excess of those reported by other workers. In this way it could reasonably be assumed that the caries inactivity was due to an innate resistance and not merely to a lack of local initiating factors. This caries inactivity was shown by two other independent strains.

The resistance may have been inherited or acquired either through the environment or through the nutritional status of the colony. The factor or factors responsible would seem to reside within the animal itself and therefore must be found either in the structure of the teeth, in the bacterial flora of the mouth, in the quantity or quality of the saliva, or in any systemic factor influencing these. However, all the efforts to modify one or another of these factors failed, except the gross alteration in the oral environment which follows loss of function of the major salivary glands. The latter resulted in massive tooth destruction, which, for the most part, was quite unlike the few carious cavities seen in the intact animals. Instead of the lesions commencing in the enamel, the latter was undermined at the amelocement junction. Whether these phenomena represent a change in the relative susceptibilities of the various tooth surfaces or whether an alteration in oral environment has produced tissue destruction of a different etiology is not known.

This is not the first report of caries resistance in hamsters. King[6] noted a resistance to dental caries in these animals, and later Mitchell and Johnson[7] could find no explanation for a caries inactivity occurring in a hamster colony previously caries-active. Keyes[8] described a caries-resistant colony originating from one female obtained from

the Rochester caries-susceptible strain, and he carried out an extensive inquiry into this apparent anomaly, which culminated in his[9] discovery of the infectious nature of the disease in these animals. His evidence of the need for establishing the correct oral flora in order to induce dental caries in rodents is a very important step forward in our understanding of this disease. It is hoped to extend the present studies further along these lines.

Thus the cause of the resistance is still unknown. It would seem, from the available evidence, to be associated with the presence of normally functioning salivary glands. Further investigations are necessary to define this more clearly.

SUMMARY

Golden hamsters of the NIMR strain possessed a marked resistance to dental caries which appeared to be unaffected by feeding a synthetic diet during tooth development, a reduction in the fluorine content of the teeth, reductions in the calcium and vitamin D contents of the diet after the eruption of the teeth, and the conditions under which the animals were kept. Two other strains of hamsters kept under similar conditions showed comparable resistance to caries.

Loss of function of the major salivary glands in animals receiving a high-sugar, synthetic diet resulted in the production of lesions commencing at the amelo-cement junction and undermining the tooth crown.

Under the conditions of this study, feeding a relatively large quantity of sugar to hamsters over a considerable period of time did not necessarily produce caries.

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Conditions Affecting Sensitivity of Techniques for Detection of Bacteremia

I. B. BENDER, SAMUEL SELTZER, GERTRUDE MELOFF, and R. S. PRESSMAN
Albert Einstein Medical Center, Philadelphia, Pennsylvania

Many difficulties are encountered in the isolation of bacteria that gain forceful entry into the blood stream during tooth removal. Blood-culture techniques must be sensitive to detect the relatively few organisms present. Contamination of the culture must be controlled; otherwise, the detection of micro-organisms may be inaccurately interpreted. This is particularly important for the detection of postextraction bacteremia because some of the organisms, which in ordinary cultures might be disregarded as contaminants, are true inhabitants of the mouth that can ultimately enter the circulation.

There is a wide variation in the percentage of positive blood cultures found after extraction of teeth as reported by different investigators; the range is from 12.8 to 93.4 per cent. A comparison of the work of various investigators revealed considerable differences in clinical and laboratory techniques, differences adequate to explain the disparate results.[1–12] Variances occurred in such clinical matters as the sterility of the gingival sulcus before tooth extraction, the number of teeth extracted at one sitting, the degree of trauma, and the kind of anesthesia. Laboratory practices varied in such matters as the size of the blood sample, the number of aliquots cultured, the addition of agents to neutralize the antibacterial action of the blood, the number of different media, and the utilization of aerobic, as well as anaerobic, culturing methods.

Whereas clinical practices affect the actual incidence of bacteremia, laboratory practices affect the success of its detection. If contamination is avoided and if clinical conditions are comparable, those laboratory procedures which yield the highest percentage of positive blood cultures are the most sensitive.

The purpose of this investigation was to determine what laboratory conditions increase the sensitivity of blood-culture technique for the detection of dental bacteremias. Four sets of conditions were varied: (1) different media and the use of aerobic and anaerobic procedures, (2) single blood samples as compared with multiple aliquots, (3) the neutralization of the antibacterial action of blood, and (4) variations in the size of the blood samples.

EXPERIMENTAL METHODS

The data of the laboratory investigations came from three experimental studies in a series of 124 cases following tooth removal. The patients ranged from sixteen to ninety

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TABLE 1*

DATA ON BLOOD CULTURES OF INDIVIDUAL PATIENTS (THIRD STUDY): EFFECT OF "LIQUOID," VOLUME OF BLOOD, AND MULTIPLE ALIQUOTS OF BLOOD ON NUMBER OF POSITIVE BLOOD CULTURES FOLLOWING EXTRACTION OF TEETH

PATIENT	NO. OF TRAUMA	IMMEDIATE GROUP					10-MINUTE GROUP				
		1		2		3	1		2		3
		BHI 4:20 Flasks		BHI+L 4:20 Flasks		BHI+L 8:40 Flask	BHI 4:20 Flasks		BHI+L 4:20 Flasks		BHI+L 8:40 Flask
		C	D	A	B	E	C	D	A	B	E
1 MK	2	6, 1	0	6, 1	6	6, 1	6	0	6	0	6
2 MM	2	0	0	1	1	1	0	0	0	0	0
3 LT	1	2	1	1	1	1	0	0	0	0	1
4 AH	1	6	0	6, 2	0	2	0	0	0	0	0
5 KA	1	3	0	0	3	3	0	0	3	0	0
6 MW	2	7	7	7	7	7	0	0	7	7	7
7 MH	2	1	2	2	1	1	0	0	1	0	1
8 MJ	2	0	2	6, 2	2	2	6	0	0	0	0
9 VW	1	0	0	0	0	0	0	0	0	0	0
10 WW	1	0	0	5, 2	2	5	0	0	0	0	0
11 BO	2	1	0	6, 1	1	6, 1	0	0	0	0	0
12 MW	2	6	1	6	6, 1	1	1	0	1	0	1
13 WW	1	7, 6	0	7, 6	7, 6, 2	7, 6	0	2	0	7, 2	0
14 SG	2	6, 1	6, 1	6, 1	6, 1	6, 1	6	6	6, 1	6, 1	6, 1
15 LL	2	5	2	0	0	5, 2	7	0	7, 5, 2	0	0
16 RB	2	6	6	6	6	6, 5	0	6	0	6	1
17 LL	2	6, 1	6, 1	2, 1	6, 2	6, 2	6	6, 2	2, 1	1	6, 2
18 ER	1	0	0	0	0	1	0	0	0	0	0
19 EP	2	1	1	2, 1	1	2, 1	1	1	1	2, 1	1
20 WL	2	1	0	1	1	1	1	0	0	0	0
21 PT	1	0	1	7	6, 1	1	0	0	0	0	0
22 HC	1	3	0	3	3	3	0	0	0	0	0
23 JS	1	1	1	1	1	1	0	0	1	1	0
24 MW	1	7, 1	1	1	7, 1	1	1	1	1	1	1
25 HW	1	0	0	0	2	0	0	0	0	0	0
26 HM	2	0	0	0	0	0	0	0	0	0	0
27 EH	1	0	0	0	0	0	0	0	0	0	0
28 EMH	2	2	1	2, 1	2	Contam.	2	2	2	0	0
29 JD	1	2	0	2	2	2	0	0	0	2	2
30 IM	2	0	0	0	6, 3	6, 7	0	0	0	0	0
31 MP	2	6	1, 2	1, 2	2	1	0	0	0	0	0
32 RT	2	0	0	6	3, 7	3, 7	0	0	2	0	2
33 JS	2	0	0	1	6	1	0	0	0	0	0
34 OJ	2	2	2	0	2	2	0	0	2	2	0
35 FP	2	7	7	7	7	7	7	0	7	7	7
36 CB	2	1	0	0	1	0	0	0	0	0	0
37 JT	2	6	6	6	1	1	0	0	1	1	1
38 ID	2	1	1	1	1	1	1	1	1	1	1
39 AW	2	1	1	1	1	1	0	1	0	0	0
40 SM	2	0	0	0	0	6	0	0	5(?)	0	0
41 JC	1	0	0	1, 7	0	0	0	0	0	0	0
42 BJ	1	0	0	0	0	0	0	0	0	0	0
43 JC	1	0	0	0	0	0	0	0	0	0	0
% Pos. 4 ml.		62.7	45.1	69.7	76.7		27.9	20.9	41.8	30.2	
% Pos. 8 ml.		67.4		81.4		80.1	34.9		48.8		37.2
% Pos. 16 ml. based on A, B, E		88.3					51.1				
% Pos. 24 ml. based on A, B, C, D, E		88.3					58.1				

* BHI—Brain-heart infusion broth
L—Sodium polyanetholsulfonate ("Liquoid")
1—*S. viridans* (group)
2—Non-hemolytic streptococcus
3—*Pneumococcus*
4—*Staph. albus hemolyticus*
5—*Staph. albus non hemolyticus*
6—Diphtheroids
7—*Flavobacterium*
0—Negative culture
Blood cultures of all patients were negative before extraction.

years of age, each requiring extraction of two or more teeth. Extractions were graded on the degree of trauma; 1 for light, 2 for heavy trauma (Table 1). All extractions were performed by a single operator in each study. The area of extraction was not isolated with cotton rolls, nor was the field sterilized. All patients were anesthetized with 2 per cent procaine hydrochloride, with epinephrine 1:50,000.

The first sample of blood was withdrawn immediately after injecting the anesthetic solution (usually about 2–5 minutes before extraction). The second sample was withdrawn immediately after extraction (usually within zero to 90 seconds). The third sample of blood was taken 10 minutes after extraction. In each instance the blood was drawn from the median basilic vein by means of a 30-ml. syringe and a 20-gauge needle.

Blood culture techniques.—In the first study 16 ml. of blood were withdrawn at one time and divided into 4 parts for culture as follows:

For aerobic study, 2 samples of 4 ml. each were inoculated in duplicate into 2 flasks with rubber diaphragms.* All flasks contained 20 ml. brain-heart infusion broth† fortified with 0.033 per cent sodium polyanetholsulfonate.‡

For anaerobic study, 2 samples of 4 ml. each were inoculated in duplicate into two 25-ml. test tubes (screw-capped with rubber diaphragms), containing 20 ml. thioglycollate medium. Sodium polyanetholsulfonate 0.033 per cent was added to the medium in 20 cases but not to another 27, in which only 2 ml. of blood were withdrawn.

In the second study, 20 ml. of blood were withdrawn at one time and divided into 2 parts as follows: Ten ml. of blood were inoculated into a rubber-stoppered flask containing 50 ml. of trypticase broth (BBR). The other 10 ml. of blood were inoculated into a flask (with rubber diaphragm) containing 50 ml. of brain-heart infusion broth fortified with 0.033 per cent of sodium polyanetholsulfonate.

In the third study, the size of the blood sample was 24 ml. divided into 5 aliquots as follows: Two 4-ml. samples of blood were inoculated into 2 flasks each containing 20 ml. of brain-heart infusion broth fortified with 0.033 per cent of sodium polyanetholsulfonate. Another two 4-ml. samples were inoculated in a similar manner without the addition of sodium polyanetholsulfonate. In the fifth aliquot, 8 ml. of blood were inoculated into a single flask containing 40 ml. of brain-heart infusion broth fortified with 0.033 per cent sodium polyanetholsulfonate.

All blood samples were incubated at 37° C. and examined at 24-hour intervals for 7 days. Cultures that became cloudy, showed color change, developed an odor, or otherwise appeared to show growth were opened and examined to identify the organisms. Specimens which were suspected of being positive were subcultured before being discarded.

The anaerobic technique followed the Brewer-Brown method.[13] A blood sample was regarded as "positive" if any one of the aliquots of that sample yielded growth.

RESULTS

First study: sensitivity of aerobic and anaerobic media.—In the blood samples taken immediately before extraction, only one was positive for growth. In the cultures

* McCartney

† Diffco®

‡ Liquoid®

taken immediately after and 10 minutes after extraction the results were as follows: In 50 cases, 40 or 80 per cent were positive in the aerobic series. In the 10-minute group, 15 out of 50, or 30 per cent, were positive (see Table 2).

In the anaerobic group in which 2 ml. were used without the addition of sodium polyanetholsulfonate, 17 out of 27 cases, or 62.9 per cent, were positive immediately after extraction. In the 10-minute blood samples, 2, or 11.1 per cent, were positive for growth. In the group in which sodium polyanetholsulfonate was added, 10 out of 20 cases, or 50 per cent, were positive in the group where cultures were taken immediately following extraction; while, in the group cultured 10 minutes after extraction, 4 out of 20, or 20 per cent, of the cultures were positive for growth.

Only in one instance was a positive culture present in the anaerobic and not in the aerobic series. The use of the anaerobic medium proved to be of value in making dif-

TABLE 2

COMPARATIVE INCIDENCE OF BACTEREMIA IN MULTIPLE EXTRACTIONS FOLLOWING USE OF VARIOUS MEDIA

STUDY*	MEDIA†	VOLUME OF BLOOD (ML.)	IMMEDIATELY AFTER EXTRACTION			10 MINUTES AFTER EXTRACTION	
			No. of Cases	No. of Positive	Per Cent Positive	No. of Positive	Per Cent Positive
1.....	B.H.I.+L	8	50	40	80.0	15	30.0
1.....	Thio.	2	27	17	62.9	3	11.1
1.....	Thio.+L.	8	20	10	50.0	4	20.0
2.....	B.H.I.+L.	10	31	23	74.2	6	20.0‡
2.....	Trypticase	10	29	11	37.9	2	7.1§
3.....	B.H.I.+L.	24	43	38	88.8	25	58.1
a.....	B.H.I.+L.	10	22	11	50.0	5	22.7
a.....	Trypticase	10	22	7	31.8	3	13.6

* *a*, From another study in which single extractions were performed.

† B.H.I. = Brain-heart infusion broth; Thio. = Thioglycolate; L = "Liquoid" (sodium polyanetholsulfonate).

‡ Based on 30 cases.

§ Based on 28 cases.

ferential counts. In 14 out of 47 cases, the anaerobic medium supported growth of different types of organisms which were not detected in the aerobic medium.

Second study: sensitivity of trypticase and fortified brain-heart infusion broth.—When cultures were taken immediately following extraction, 23 out of 31 cultures, or 74.2 per cent were positive in brain-heart infusion medium fortified with sodium polyanetholsulfonate, whereas in the trypticase medium 11 out of 29, or 37.9 per cent, were positive. Of the blood samples taken 10 minutes after extraction and inoculated into brain-heart infusion broth, 6 out of 30, or 20.0 per cent, were positive, whereas those cultured in trypticase broth, 2 out of 28 cases, or 7.1 per cent, were positive (Table 1).

Third study: (a) Sensitivity of single blood samples and multiple aliquots.—A blood sample of a given size can be put into a single flask for culture, or it can be divided into two or more aliquots, each of which is cultured in a separate flask.

An 8-ml. blood sample was cultured in a single flask (E) containing 40 ml. of brain-

heart infusion broth. The other 8 ml. were divided into two 4-ml. aliquots (flasks A and B) in duplicate culture, each flask contained 20 ml. of the same medium.

The results in 43 tests showed that both methods were equally successful in detecting bacteremia: 81.4 per cent positive for the duplicate cultures taken immediately after extraction, as compared with 80.1 per cent for single 8-ml. blood samples. The results for blood samples taken 10 minutes after extraction were 48.8 per cent positive for duplicate cultures and 37.2 per cent positive for single cultures. This difference, however, was not statistically significant.

The results suggest that the same total volume of blood is equally successful for detecting bacteremia if there is a heavy influx of micro-organisms into the blood stream as represented by the immediate group. If the bacteremia is sparse, as represented by the 10-minute group, then two small samples of blood *may* be better than a single sample of the same volume of blood for the detection of bacteria. This observation was also made by Robinson and his group.[9]

(*b*) *The neutralization of the antibacterial action of blood.*—The antibacterial action of blood in vivo has been shown in another study in which positive blood cultures were reduced from 84.9 to 44 per cent, 10 minutes after the onset of bacteremia. This antibacterial action likewise operates in vitro while the blood is being cultured. Blood remains actively phagocytic for 24 hours.[2] Therefore, false negative cultures can be obtained unless the antibacterial action of the blood is neutralized. This is particularly so if small numbers of bacteria are suspended in blood.

In this investigation, sodium polyanetholsulfonate was added to the blood culture flasks for the purpose of inhibiting the antibacterial action of the blood. It is a powerful anticoagulant,[15] inactivates the blood complement,[16] and also interferes with the phagocytic activity of leukocytes.[17] In experimental tissue cultures, traces of sodium polyanetholsulfonate completely prevented phagocytosis; at concentrations as low as 0.01 per cent it inhibited leukocyte migration.[18]

Blood cultures were made both with and without the addition of sodium polyanetholsulfonate in 43 cases. Bacteremia was detected in 81.4 per cent of the cases with the use of the drug but in only 67.4 per cent of the cases without the use of this drug. The preceding results are for blood taken immediately after tooth extraction; the results for blood samples taken 10 minutes after extraction are 48.8 per cent with sodium polyanetholsulfonate as against 34.9 without the addition of the drug. These figures indicated that the addition of sodium polyanetholsulfonate increased the sensitivity of the blood culture techniques. This is also corroborated by the results obtained with the use of 4-ml. blood samples (Table 2).

(*c*) *Effect of sample size.*—In a slight bacteremia, such as is common after extraction, it is conceivable that a large sample of blood would contain enough organisms to produce a positive blood culture, whereas a small sample might or might not show up as positive, depending on the chance distribution of the few organisms.

Since some of the blood samples were divided into two aliquots of 4 ml. each, the incidence of bacteremia could be assessed for each 4-ml. sample. In one of the 4-ml. samples, 30 out of 43 cases, or 69.7 per cent, were positive, while in the second 4-ml. sample, 33 out of 43 cases, or 76.7 per cent, were positive. Therefore, the incidence of bacteremia could vary from 69.7 to 76.7 per cent, or an average of 73.2 per cent for each 4-ml. sample of blood (Table 3, first and second columns).

The results of the culture of 8 ml. of blood were obtained by combining the results obtained from two 4-ml. aliquots (flasks A and B) and considering it as positive if either of the two flasks was positive. It was found that 81.4 per cent yielded positive cultures. When 16 ml. of blood were cultured by combining 3 aliquots (two 4-ml. samples and one 8-ml. sample, flasks A, B, and E), the percentage of positive increased to 88.3 per cent. When a 24-ml. blood sample was cultured, using five aliquots (flasks A, B, C, D, and E), immediately after multiple tooth removal, the percentage of positive cultures was the same, 88.3 per cent. It was therefore concluded that a 16-ml. sample of blood was an optimum quantity for use in determining the presence of micro-organisms in the blood stream immediately following tooth removal.

In the 10-minute blood sample following extraction, the incidence of bacteremia also increased as the blood sample size was increased. The 4-ml. samples of blood were positive in one group, 30.2 per cent, and in the other 41.8 per cent, or an average of 36 per cent. The 8-ml. blood sample was 48.8 per cent; 16 ml. of blood was 51.1 per cent; and 24 ml. of blood was 58.1 per cent positive (see Tables 2 and 3).

TABLE 3

EFFECT OF BLOOD VOLUME, LIQUOID, AND MULTIPLE ALIQUOTS ON NUMBER OF POSITIVE BLOOD CULTURES IN 43 CASES

Volume of Blood (Ml.)*	No. of Aliquots	Percentage of Positive		Volume of Blood (Ml.)*	No. of Aliquots	Percentage of Positive	
		Immediate	10 Minutes			Immediate	10 Minutes
4	Single	53.9†	24.4†	8+L	Single	80.1	37.2
4+L	Single	73.2†	36.0†	16+L	Three	88.3	51.1
8	Two	67.4	34.9	24+L	Five	88.3	58.1
8+L	Two	81.4	48.8				

* L = Sodium polyanetholsulfonate ("Liquoid").
† Average for two 4-ml. samples.

The results for this series of experiments thus indicated that, for blood samples taken immediately after extraction, increasing sample size to 16 ml. of blood increased the sensitivity of detection. For blood samples taken 10 minutes after extraction, 24 ml. gave a higher incidence of positive cultures than did 16 ml.

DISCUSSION

These studies indicate that brain-heart infusion broth fortified with sodium polyanetholsulfonate (0.033 per cent) supports the growth of a wide spectrum of microorganisms and is predominantly capable of growing *Streptococcus viridans*. Still better is the use of several media.[19] While the anaerobic media did not increase the percentage of bacteremia, it did increase the detection of different varieties of organisms. The difference in the results between aerobic and anaerobic media without the sodium polyanetholsulfonate may be ascribed to the fact that only 2 ml. of blood were cultured anaerobically, while 8 ml. were cultured aerobically. The addition of sodium polyanetholsulfonate to thioglycollate under anaerobic conditions is therefore contraindicated. With the use of the drug the streptococci were isolated in 15 per cent of the cases, whereas without the drug they were present in 55.5 per cent of the cases. Per-

haps, as suggested by Hoare,[20] sodium polyanetholsulfonate inhibits growth of anaerobic streptococci.

Trypticase broth, under the conditions of the test, was not found to be a sensitive medium, nor did it increase the detection of different micro-organisms that were found in the fortified brain-heart infusion broth.

The micro-organisms recovered in studies 1 and 3 are presented in Table 4. Table 5 shows the bacteria that were recovered in study 2.

Some investigators regard the presence of bacteria other than streptococci as contaminants. In the authors' opinion, any bacteria that are present in the mouth can gain entry into the circulation if there is sufficient trauma. This is especially true if the micro-organisms are present in large numbers at the place of entry. Streptococci are found frequently in the blood after tooth extraction, not because they have any special priority for entering the circulation, but because they are the most common and usually the most numerous inhabitants of the mouth. Streptococci are not always

TABLE 4

ORGANISMS RECOVERED FROM BLOOD IN FIRST AND THIRD STUDIES

ORGANISM*	IMMEDIATELY AFTER EXTRACTION		10 MINUTES AFTER EXTRACTION	
	Aerobic 93 Cases	Anaerobic 47 Cases	Aerobic 93 Cases	Anaerobic 47 Cases
S. viridans (group)	51 (55.9%)	17 (36.1%)	24 (26.0%)	2 (4.2%)
S. non hemolyticus	19 (20.4%)	11 (23.4%)	10 (10.7%)	4 (8.5%)
Pneumococcus	8 (8.6%)	1 (2.1%)	0	0
Staph. albus hemolyticus	2 (2.1%)	0	1 (1.0%)	0
Staph. albus non hemolyticus	12 (12.8%)	3 (6.3%)	5 (5.3%)	3 (6.3%)
Diphtheroids	15 (16.2%)	0	5 (5.3%)	1 (2.1%)
Flavobacterium	8 (8.6%)	0	4 (4.3%)	0

* Some positive cultures had combinations of two or more organisms. *Staph. albus non hemolyticus* was recovered once in one of the blood samples before extraction.

TABLE 5

ORGANISMS* RECOVERED FROM BLOOD IN SECOND STUDY

ORGANISM	IMMEDIATELY AFTER EXTRACTION		10 MINUTES AFTER EXTRACTION	
	B.H.I.	Trypticase	B.H.I.	Trypticase
S. viridans (group)	13	6	3	0
Staph. albus hemolyticus	2	2	0	1
Diphtheroids	9	3	3	1
Neisseria	2	0	0	0
S. non hemolyticus	1	0	0	0
Staph. aureus	1	0	0	0
Micrococcus	0	1	0	0
Staph. albus non hemolyticus	2	1	0	0
Lactobacillus sp.	1	0	1	0
B. subtilis	1	0	0	0

* More than one organism was recovered in each flask.

the most numerous; bacterial population counts of the oral cavity in over 300 patients showed that in many mouths the predominant bacteria were staphylococci; in some instances there was a high count of diphtheroids and flavobacteria. Burn and Burket[21] in postmortem studies of the periapical tissues and the blood stream showed the presence of staphylococci in 48 per cent of the positive cultures obtained from the tissues and in 42 per cent of the positive cultures obtained at the same time from the blood stream. Staphylococci, pneumococci, diphtheroids, and other organisms are inhabitants of the oral cavity and can gain access to the circulatory system.

The technique utilized in this study was rigidly controlled to prevent contamination. The following facts are offered in evidence: (*a*) Only one patient had a positive blood culture before tooth removal. It is possible that even this may not have been a contaminant. If contamination was a factor, then the preoperative cultures would have been positive more often. (*b*) Only once was a positive culture present in the 10-minute series that was negative in the immediate series. (*c*) In almost every instance the bacteria found in the blood 10 minutes after extraction were the same as those found in the blood taken immediately after extraction.

The question arose as to whether comparison of results in the third study was permissible, inasmuch as a single 24-ml. blood sample was divided into three parts for study, and differences in the results for the three parts might have been due to differences in the chance distribution of bacteria. However, results of the third study corresponded closely with those of the first study: 8-ml. blood samples in the first study were 82 per cent positive; 8-ml. samples in the third study were 81.4 per cent positive. There was also another check within the third study: 8 ml. in two aliquots, 81.4 per cent; 8 ml. in a single flask, 80.1 per cent. It may be concluded from this study that bacteremias occur in all patients following extraction. Their detection is dependent on the size of the blood sample and the refinement of technique.

Since the bacteremias in postextraction and subacute bacterial endocarditis are quite similar, the techniques used in this investigation may be fruitful in detecting bacteria in patients suffering from subacute bacterial endocarditis.

SUMMARY

Extraction of teeth causes bacteremias which may be difficult to detect by the usual blood culture techniques because the number of bacteria in the blood stream is small. By varying conditions of blood cultures in 124 cases, the following results were obtained:

1. Sensitivity of blood-culture techniques for the detection of bacteremia was increased by adding an inhibitor, sodium polyanetholsulfonate, to neutralize the natural antibacterial activity of the blood.

2. The use of more than one culture medium increased the sensitivity of the blood-culture techniques.

3. The use of an anaerobic along with an aerobic medium made possible the detection of a greater variety of bacteria in the blood.

4. The use of a greater volume of blood for cultures produced an increase in the detection of bacteremia.

5. For a given volume of blood, the sensitivity was the same whether the entire sample was cultured in a single flask or divided into two or more aliquots for separate

culturing, when the bacterial showers were heavy. When the bacteremia was sparse, two or more aliquots may have been better.

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Dentinal and Pulpal Response. V. Application of Pressure to Exposed Dentin

MARTIN BRÄNNSTRÖM
Department of Dental Histopathology, Royal School of Dentistry, Stockholm

The view has been advanced that occasional pain and inflammatory changes experienced in the pulp are due primarily to pressure during application of filling material.[1] It has also been suggested that odontoblasts might move into the dentinal tubules as an indirect result of pressure on the open tubules.[2, 3] A survey of the reports in which this reaction is discussed has been presented in an earlier paper.[4] It has since been suggested that pressure applied during drilling or by modeling-compound impression may give rise to a loss of odontoblasts through their migration into the tubules[5] and also be the cause of inflammatory reaction in the pulp.[6] No histologic examinations have been published indicating what effect pressure on the exposed dentin might have on the pulp.

The dentin is usually considered not to contain nerve fibers, and the reason for the sensibility of this tissue is therefore obscure. According to one theory, the contents of the tubules might transmit pressure from the periphery of the tubules to the pulp and thereby give rise to pain.

The purpose of this study was to investigate the effect of applying pressure to exposed dentin and to establish whether pain is elicited and histologic changes produced in the odontoblast layer and the adjacent region of the pulp.

EXPERIMENTAL METHODS

The pressure was obtained with air or water, and the experiments were divided into four groups: (1) application of air pressure—1 kg/sq cm for 2 minutes; (2) application of air and water pressure until pain was reported; (3) application of air and water pressure—2 kg/sq cm for 3 minutes; (4) application of water pressure—2 kg/sq cm for 3 minutes. The teeth were extracted after one week.

Group 1: Application of air pressure—1 kg/sq cm for 2 minutes.—Buccal cavities, 2 mm. in diameter and about the same in depth, were prepared in 7 contralateral pairs of premolars with intact buccal surfaces and obtained from subjects aged twelve to eighteen years. The method was that used in earlier experiments[4, 7–9] and has been described in detail elsewhere.[4] One cavity was chosen from each pair at random, was rinsed with water at 37° C., and dried with cotton pellets. The cavity was then subjected to air pressure of 1 kg/sq cm for 2 minutes, after which a moist pellet was inserted. In the meantime a moist pellet was placed in the cavity of the contralateral tooth—the

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control. After ½–1 minute a local anesthetic* (2 per cent) was administered and 5 minutes later both teeth were extracted.

The experiment was performed with the rubber dam in place. Prior to the application of the pressure, the patient was instructed to indicate when pain was felt and whether it diminished or ceased. The pressure was supplied from a cylinder of compressed air with a back-pressure valve and a manometer. A rubber hose from the valve was connected to a metal tube 1.9 mm. in external diameter. The end of the tube was supplied with a rubber washer and placed against the cavity aperture. The washer was attached to the enamel surface around the cavity in such a way that no leakage

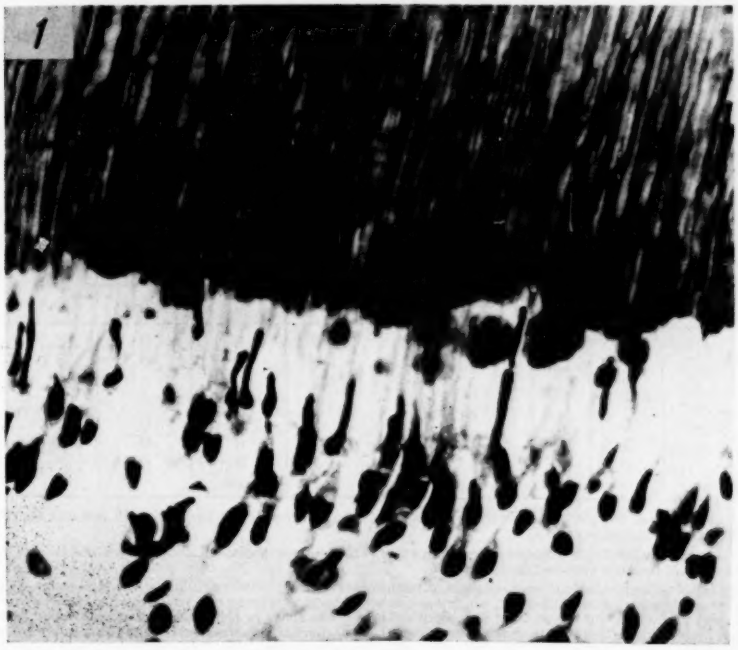

FIG. 1.—Dentin and pulp beneath a cavity exposed to air pressure of 1 kg/sq cm for 2 minutes. Odontoblast nuclei in the dentinal tubules (Pair 6). ×430.

could be observed during the application of the pressure. Before pressure was applied, a check was made to ascertain that the tube itself did not cause pain.

The preparation of the histologic specimens has been described earlier.[4] The histologic examination was performed without knowledge of the clinical data.

Pain was felt in the case of one of the seven teeth. It lasted throughout the period that the pressure was applied but diminished toward the end. In no instance was pain reported just after the pressure was discontinued.

Histologic examination revealed odontoblast nuclei in the tubules beneath all the cavities subjected to pressure (Fig. 1). In four cases, however, the number of nuclei in the tubules was small. This reaction was not observed under any of the control activities (Table 1).

* Xylocaine—Norxadrine.

In addition to these findings, hyperchromasia was observed under some of the test and control cavities but only in the area of the blastema. In a few test and control teeth, there was also an eosinophilic area or extravasated erythrocytes in an area of the pulp beneath the cavity. Under all the cavities the pulpo-dentinal membrane was interrupted. Under none of the test or control cavities were there vesicles.

Group 2: Application of air and water pressure until pain was felt.—Experiments were performed on ten premolars with intact buccal surfaces from subjects aged ten to seventeen years. The cavities were prepared in the same way as in Group 1. Five cavities were subjected to an initial air pressure of 0.5 kg/sq cm, which was slowly increased until pain was reported (increase from 0.5 to 2 kg/sq cm over a period of

TABLE 1

CLINICAL AND HISTOLOGIC OBSERVATIONS UNDER CAVITIES OF 7 CONTRALATERAL PAIRS OF PREMOLARS*

Pair No.	Distance to Pulp† (Mm.)		Odontoblast Nuclei in Tubules‡				Duration of Pain
			Test Tooth		Control Tooth		
	Test	Control	0-Section	Other Sections	0-Section	Other Sections	
1.....	0.8	0.9	120	++	0	0	.....
2.....	1.9	2.0	10	0	0	0	.....
3.....	2.0	1.6	15	+	0	0	.....
4.....	1.7	1.3	60	+	0	0	.....
5.....	0.6	1.0	30	+	0	0	.....
6.....	1.9	1.7	160	++	0	0	2 minutes
7.....	0.9	1.1	250	++	0	0	.....

* The cavity of one tooth, the test tooth, was subjected to air pressure of 1 kg/sq cm for 2 minutes; the contralateral tooth served as control.

† The recorded distance from the floor of the cavity to the pulp was measured along the dentinal tubules and is the shortest distance observed in the sections.

‡ Classification of the number of odontoblast nuclei observed in the dentinal tubules: *0-section*—one section, chosen from the 90 or so of each tooth, which was the most nearly parallel section to the dentinal tubules under the cavity and which had the thinnest pulpodentinal membrane above or below the area corresponding to the cavity. It is in this section that the nuclei were counted. The other sections were classed according to the following system:

0 = No, or very few, odontoblast nuclei in the dentinal tubules.
\+ = Few odontoblast nuclei (approx. 5–50) in the dentinal tubules.
++ = A moderate number of odontoblast nuclei (approx. 50–200) in the dentinal tubules.

20–30 seconds). When pain was felt, the pressure was maintained constant for about 10 seconds, after which it was discontinued.

In order to see whether the same histologic changes were obtained with water pressure, the same pressure of water at 37° C. was applied to another five cavities; the method was otherwise identical. The water pressure was obtained by fluid transmission from a container connected to the air-pressure tube. In order to avoid the inclosure of air bubbles in the cavities, the water was allowed to flow round the cavity before the tube was fixed in place. The method and apparatus were otherwise as for Group 1.

Pain was felt with air pressure in all five cases tested, and with water pressure in four out of the five cases when the pressure rose to between 2 and 3 kg/sq cm. The pain ceased when the pressure was discontinued. In one case, where water pressure

was used, no pain was reported when the pressure rose to 3 kg/sq cm, at which level the experiment was discontinued.

Histologic examination revealed vesicles of varying appearance under all the cavities subjected to air pressure, and under one of the cavities there were a few odontoblast nuclei in the tubules. Under the cavities subjected to water pressure, there were no vesicles, but in one case there were a few nuclei in the tubules (Table 2).

Group 3: Application of air and water pressure—2 kg/sq cm for 3 minutes.—The experiment was performed on sixteen single teeth—fifteen premolars and one incisor—from subjects aged eleven to sixteen years. Cavities were prepared in the intact

TABLE 2

HISTOLOGIC OBSERVATIONS UNDER CAVITIES SUBJECTED TO AIR OR WATER PRESSURE, INCREASED FROM 0.5 KG/SQ CM UNTIL PAIN WAS ELICITED*

Case No.	Distance to Pulp (Mm.)	Odontoblast Nuclei in Tubules		Other Findings	Pressure at Which Pain Was Felt (Kg/Sq Cm)
		0-Section	Other Sections		
	Air Pressure				
8	2.1	0	0	Vesicle formation	2.4
9	1.6	0	0	Vesicle formation	2.8
10	0.8	0	0	Vesicle formation	2.0
11	1.4	20	+	Vesicle formation	3.0
12	1.8	0	0	Vesicle formation	2.5
	Water Pressure				
13	1.0	0	0	.....	2.8
14	1.9	0	0	.....	(pressure interrupted at 3.0)
15	1.0	45	+	.....	2.8
16	1.7	0	0	.....	2.8
17	1.5	0	0	.....	2.0

* See Table 1 for explanation of entries.

buccal surfaces as in Groups 1 and 2. To eight of the cavities, air pressure at 2 kg/sq cm was applied for 3 minutes. The other eight cavities were subjected to water pressure at the same level for 3 minutes. The method was otherwise the same as for Groups 1 and 2.

Pain was felt only in one case when air pressure was applied. The pain was reported after the pressure had been applied for 1½ minutes, and it persisted until the 3-minute period had elapsed. Water pressure produced pain in all teeth tested—immediately in seven cases, and after 1 minute in one case. With one exception, the pain was described as diminishing, and it ceased within ½–1½ minutes. In one case the pain increased, and the pressure was therefore discontinued after 1 minute.

Histologic examination revealed nuclei in the tubules under five out of the eight

cavities exposed to air pressure, and in three of these there were only a few. Vesicles were found under four cavities (Figs. 2–4).

Beneath the cavities exposed to water pressure there were a few nuclei in the tubules of three teeth. In no case were vesicles observed in the pulp (Table 3).

Group 4: Application of water pressure—2 kg/sq cm for 3 minutes. Teeth extracted after one week.—Buccal cavities were prepared in nine intact contralateral pairs of premolars from subjects aged eleven to fifteen years. The cavities were 2 mm. in diameter and about 2 mm. in depth. One cavity of each pair was chosen at random and subjected to water pressure—2 kg/sq cm for 3 minutes. The other cavity, the control, was kept wet. Both cavities were then dried with pellets and filled with amalgam. The teeth were extracted after one week.

Pain was felt in seven out of nine cases and ceased after a period of between 20 seconds and 3 minutes. In two cases (Nos. 33 and 34) no pain was felt.

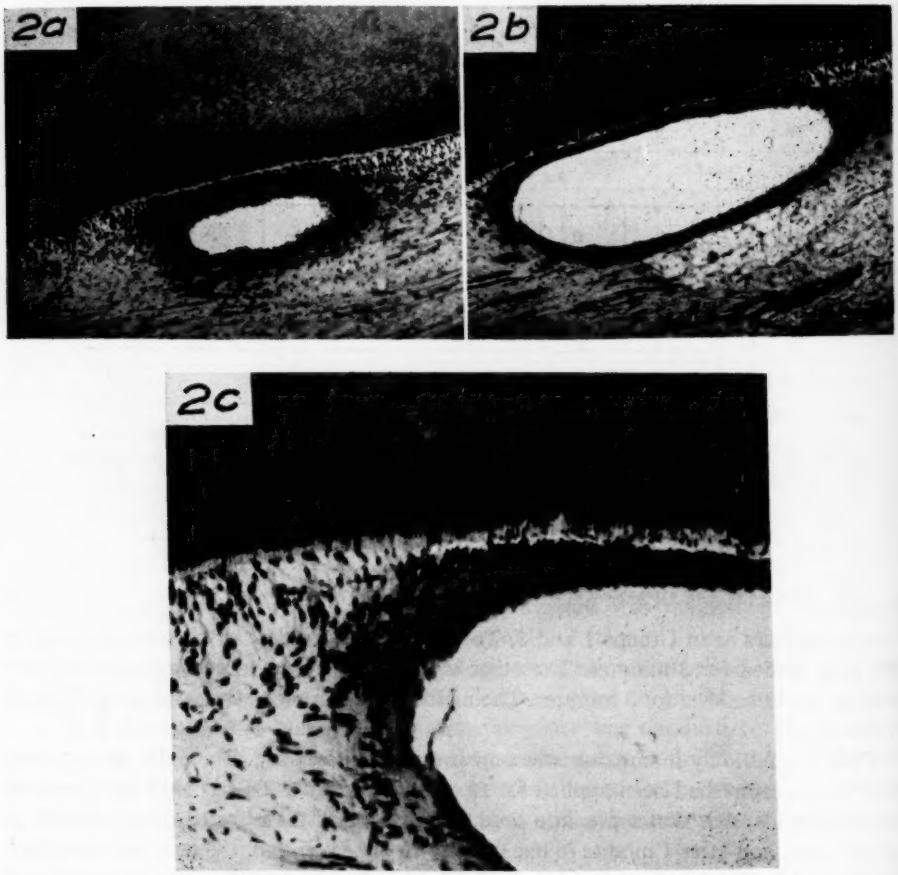

FIG. 2.—Dentin and pulp beneath a cavity exposed to an air pressure of 2 kg/sq cm for 3 minutes (Case 19). *a*, Vesicle formation, ×48; *b*, another section from the same tooth, ×48; *c*, higher magnification of the left area in Fig. 2, *b*, odontoblast nuclei in the tubules, ×160.

FIG. 3.—Vesicle formation in the pulp beneath cavity exposed to air pressure of 2 kg/sq cm for 3 minutes (Case 18). ×48.

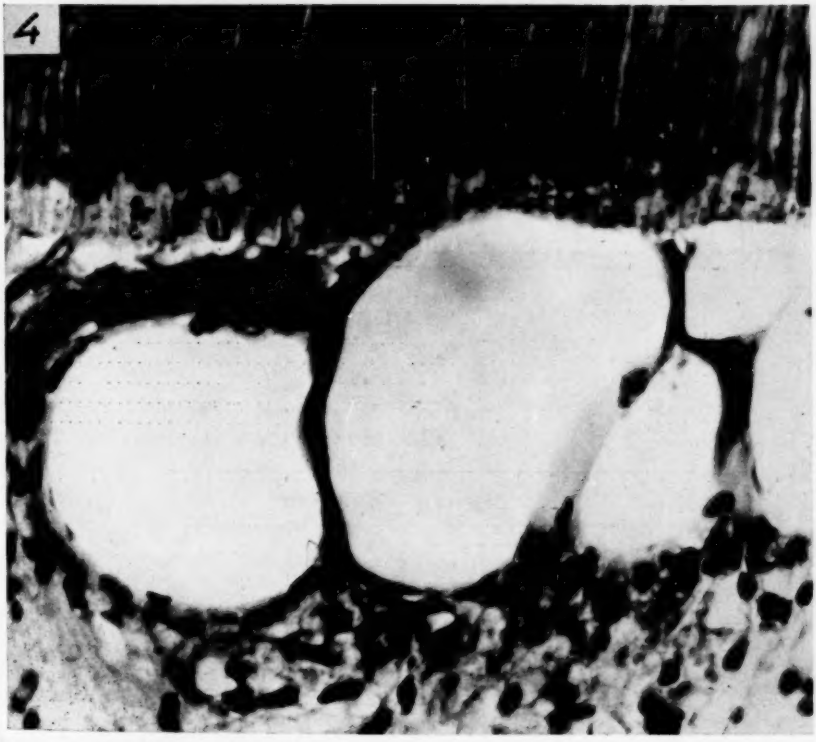

FIG. 4.—Vesicle formation in the pulp beneath cavity exposed to air pressure of 2 kg/sq cm for 3 minutes (Case 25). ×430.

Histologic examination revealed that beneath all test cavities there was a marked reduction of the odontoblast layer and odontoblast nuclei, or nuclei remnants were present in the corresponding dentinal tubules (Fig. 5). This change was observed beneath two control cavities but only to a slight degree. A reduction of the predentin zone had occurred beneath seven test cavities and one control cavity. A small number of leucocytes was found at the predentin border under all test cavities and three control cavities (Table 4). All changes observed were limited to the area beneath the cavity.

DISCUSSION

Displacement of odontoblasts.—For those experiments in which the teeth were extracted a few minutes after air or water pressure had been applied, it was noticed that beneath only five cavities exposed to air pressure were there more than a few nuclei in the tubules. This finding might be ascribed in part to reactions taking place after the exposure to air pressure. During the application of pressure, air would be dissolved in the contents of the tubules, and this might have resulted in liberation of bubbles when the pressure was reduced. This in turn may have caused an outward movement of the contents of the tubules toward their apertures in the cavity.

TABLE 3*

CLINICAL AND HISTOLOGIC OBSERVATIONS UNDER CAVITIES SUBJECTED TO AIR OR WATER PRESSURE, 2 KG/SQ CM FOR 3 MINUTES

CASE NO.	DISTANCE TO PULP (MM.)	ODONTOBLAST NUCLEI IN TUBULES		OTHER FINDINGS	ONSET AND DURATION OF PAIN
		0-Section	Other Sections		
	Air Pressure				
18	0.9	20	+	Vesicle formation	
19	1.1	260	++	Vesicle formation	
20	1.6	30	+		
21	1.5	120	++		
22	1.9	0	0		
23	1.3	15	0	Vesicle formation	
24	1.7	0	0		
25	0.6	0	0	Vesicle formation	After 1½ min., continuous
	Water Pressure				
26	1.7	50	+		After 1 min., subsiding after 1 min.
27	1.2	15	+		Immediate, subsiding after 30 sec.
28	1.2	0	0		Immediate, increasing†
29	1.6	0	0		Immediate, subsiding after 1 min.
30	1.4	0	0		Immediate, subsiding after 1 min.
31	1.5	0	0		Immediate, subsiding after 1½ min.
32	0.9	40	+9		Immediate, subsiding after 1 min.
33	1.7	0	0		Immediate, subsiding after 1 min.

* See Table 1 for explanation of entries.
† Pressure removed after 1 minute.

It might be of interest to compare these results with those obtained in earlier experiments in which the dentin was exposed to reduced pressure or an air-stream for 2 and 5 minutes, respectively.[4, 7] Under the prevailing experimental conditions, numerous nuclei were found in the tubules beneath all the test cavities. These changes, which were observed to be an immediate result of the test applied, were ascribed to an evaporation of moisture from the orifices of the tubules, accompanied by an outward flow of the tubular contents and involving odontoblasts and sometimes also erythrocytes.

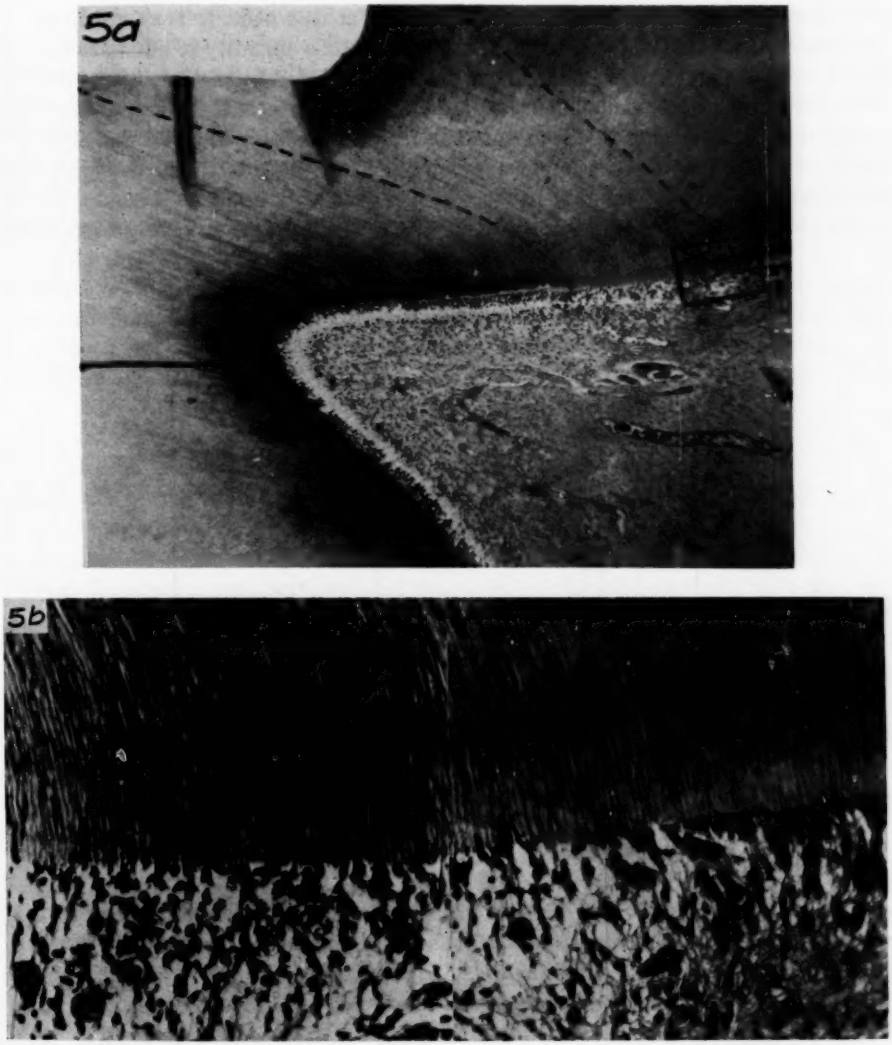

FIG. 5.—*a*, Dentin and pulp beneath a cavity exposed to water pressure, 2 kg/sq cm for 3 minutes, 1 week before extraction (Pair 38). ×32. *b*, Detail: Odontoblast nuclei in the tubules and a marked reduction of the odontoblast layer. A small number of leucocytes at the predentin border. ***Right***, unaffected area (Pair 38). ×286.

The results from Groups 2 and 3, especially from experiments with water pressure, presented no clear evidence that pressure on exposed dentinal tubules, as an immediate "aftereffect," would consistently cause the odontoblasts to move into the tubules. However, the results of Group 4 showed that the water pressure of 2 kg/sq cm for 3 minutes gave rise to a pronounced secondary movement of odontoblast nuclei into the dentinal tubules (Fig. 5). This was probably due to a later change in the contents of the tubules and the pulp.

Vesicle formations of varied appearance (Figs. 2–4) were observed under the cavities that had been exposed to high air pressure (Tables 2 and 3). The most likely explanation of this is that, owing to the pressure, a greater quantity of gas was dissolved in the tubule contents and the adjacent pulp (Henry's law) and that, when the pressure was removed, this amount of gas was liberated and formed vesicles. The process would thus resemble that shown to cause air emboli ("bends") in divers and airmen. A similar formation of vesicles has been observed in the pulp in connection with aerodontalgia,[10] under acrylic fillings,[11] and as a result of applying heat to the exposed dentin.[12] It should be mentioned in this connection that in poorly vascularized

TABLE 4*

HISTOLOGIC OBSERVATIONS ON THE DENTIN AND PULP BENEATH CAVITIES IN 9 CONTRALATERAL PAIRS OF TEETH†

Case	Cavity	Distance to Pulp (Mm.)	Reduction of Predentin	Odontoblast Nuclei in Tubules	Reduction of Odontoblast Layer		Exudate Cells	
					Slight	Marked	Lymphocytes	Leucocytes
34.....	Test	2.0	+	++		+		1
	Control	1.7						
35.....	Test	2.2	+	+++		+		1
	Control	1.6	+	+	+			
36.....	Test	1.2	+	+++		+		1
	Control	0.9						
37.....	Test	0.6		++		+	1	2
	Control	0.9						
38.....	Test	1.5	+	++		+	1	2
	Control	1.6						1
39.....	Test	0.6		++		+		2
	Control	1.3						
40.....	Test	1.1	+	++		+		2
	Control	0.8						
41.....	Test	0.9	+	++		+	1	2
	Control	1.2		+	+		1	1
42.....	Test	1.3	+	++		+		2
	Control	1.0						1

* See Table 1 for explanation of entries. Cells of exudate: 1–2 denotes slight infiltration; 3 denotes moderate infiltration; 4 denotes marked infiltration.

† The test cavity of each pair was exposed to water pressure—2 kg/sq cm for 3 minutes. Observation period one week.

tissue, especially that which absorbs large quantities of air—for instance, lipoids—a rapid reduction in the pressure or a rise in temperature may result in the appearance of gas bubbles in the tissues.

Later changes in the pulp.—As far as these can be judged from Group 4 with the observation period of 1 week, the results indicated that the later changes due to water pressure (2 kg/sq cm for 3 minutes) were essentially the same as those observed beneath cavities exposed to a continuous air stream one week before extraction. These changes have been discussed in an earlier publication.[8]

The consistent marked reduction of the odontoblast layer was probably a result of the secondary movement of odontoblasts into the tubules, and the continuous process might well be a formation of irregular secondary dentin. The fact that beneath all the cavities exposed to pressure there was also a small number of leucocytes at the predentin border, but that these were present beneath only three of the controls, suggests that the pressure gave rise to an inflammatory reaction (Table 4). The findings in Group 4 suggest that in operations in dentin, pressure—provided it acts on the contents of the tubules—might be one of the factors responsible for changes in the pulp under the dentin area involved.

The pressure at which pain was reported in Group 2 could be estimated only approximately, and an exact value of the pain threshold was probably not obtainable with a continuous increase in pressure. The observation of pain was highly subjective; moreover, there was a variety of factors such as distance to the pulp and the situation in the tubules or at the apertures that may have been responsible for individual variations.

The sensitivity of the dentin manifested in, for instance, probing or cavity preparation might arise through the transmission of pressure to the pulp by the contents of the dentinal tubules. On application of a pressure of 3 kg/sq cm to the floor of a cavity 0.03 sq. cm. in area (diameter 2 mm.), the force was 0.09 kg. Application of a probe to an area of 0.001 sq. cm. with a force of 0.1 kg. may be considered as roughly equivalent to a pressure of 100 kg/sq cm.

In earlier studies, pain was produced consistently when the exposed dentin was subjected to an air blast or reduced pressure, and in all cases histologic examination revealed odontoblast nuclei in the dentinal tubules, indicating an outward displacement of their contents during the experiments.[4, 7] As pointed out by Kramer,[13] it is probably true that odontoblasts can move into the tubules without causing pain. Since the way in which the contents of the tubules move and their speed might be critical factors in the production of pain, the possibility that the pain is elicited by a hydrodynamic mechanism and a change in pressure at the pulpal end of the tubules cannot be ruled out.

SUMMARY

The response to pressure on exposed dentin has been studied in four series of experiments. The pressure at which pain was elicited was recorded and the reaction in the dentin and pulp examined histologically. The pressure was applied with air or water at 1–3 kg/sq cm. In three series of experiments the teeth were extracted a few minutes after the pressure had been applied. In one series on contralateral pairs of teeth, one cavity of each pair was subjected to water pressure—3 kg/sq cm for 3 minutes; both cavities were filled with amalgam, and the teeth were extracted after one week.

The results of histological examination indicated that a pressure of 2–3 kg/sq cm,

provided that it is not exerted by gas, did not give rise to immediate pulp changes to any appreciable extent. However, water pressure of 2 kg/sq cm for 3 minutes resulted in a marked reduction of the odontoblast layer beneath the cavity through a secondary movement of odontoblast nuclei into the tubules. This pressure might also have been responsible for the slight inflammation observed.

Pain was elicited in most cases with a pressure of 2–3 kg/sq cm. The observation of pain in both this study and the earlier experiments seems to support the theory that the sensitivity of the dentin is dependent on a hydrodynamic or pressure mechanism.

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Carbonic Anhydrase in Dental Tissue and the Effects of Parathyroid Hormone and Fluoride on Its Activity

KENICHI KONDO and KANAME KURIAKI

Department of Pharmacology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan

The inorganic portion of tooth and bone substance very closely resembles hydroxyapatite. Recently, however, Nebergall and Kühl[1] demonstrated that when dental enamel is ignited, carbonate and pyrophosphate disappear in a molar ratio of one-to-one to produce β-$Ca_3(PO_4)_2$ and that carbonatoapatite and $CaHPO_4$ also react, when heated at 600° C., to produce the same product, β-$Ca_3(PO_4)_2$. They concluded, therefore, that dental enamel contains carbonatoapatite and hydroxyapatite. Analysis of their inorganic components, in fact, has shown that the main components may be expressed as calcium phosphate (74.6 per cent) and calcium carbonate (10.4 per cent), but the carbonate is assumed to be held on the surface of the apatite crystal and not to be a component of its lattice.[2]

On the other hand, the tissues are known to have a markedly high content of phosphatase; this enzyme is considered to catalyze the release of phosphate from its organic compounds, then the released phosphate precipitates as inorganic salt.[3] The phosphatase, however, cannot account for carbonate. If the carbonate were an essential component of the tooth and bone, the tissues should show carbonic anhydrase activity, but this matter seems hardly to have been taken up for study to date. It has been observed that the hen, given sulfanilamide, an inhibitor of carbonic anhydrase, lays eggs with very soft shells.[4] Upon examining dental tissues for carbonic anhydrase activity, we were able to establish its presence. We have investigated the effect of fluoride on the enzyme and its interaction with parathyroid. For the sake of comparison, the activity of alkaline phosphatase in dental and bone tissues and its effect on this enzyme have also been studied.

MATERIALS AND METHODS

Male albino rats weighing 200–250 gm. were used as experimental animals. Their incisors and femurs were taken out, freed of adjacent tissues, ground with 0.2 M phosphate buffer solution, and subjected to centrifugation. An aliquot of the supernatant was tested for enzyme activity. Non-carious human teeth extracted from the permanent dentition for various reasons were also tested. They were prepared in the same way as described above.

Acetazolamide* and fluoride were added in vitro; the former was also administered subcutaneously in the daily dose of 10 mg/kg dissolved in 3.5 per cent polyvinylpyrrolidon. Parathyroid extract† was injected intramuscularly in daily doses of 50 units for 10 days.

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* Diamox, Lederle Laboratories.

† Kakerubin, Mikasa Pharmaceutical Co.

For parathyroidectomy, the skin was cut open under anesthesia at the mid-line of the ventral side of the neck to expose the sternohyoid muscle. When the muscle was separated along the mid-line, the thyroid gland could be located easily. The parathyroid—a tiny, round body the size of a poppy seed and of yellowish hue, different from the purple thyroid—lies on the anterolateral surface of each lobe of the thyroid. It could be removed easily. The operated rats were allowed to recuperate for 15 days before they were used in the experiments.

Determination of carbonic anhydrase (CAH) activity was performed manometrically, following the method of Altschule and Lewis[5] and of Mitchell;[5] the activity was expressed in Kd units per gram tissue.

The alkaline phosphatase (APH) activity was determined according to the method of Shinowara, Jones, and Reinhart[6] and expressed as the amount (mg.) of phosphate liberated per gram tissue.

RESULTS

The results are given in Tables 1 and 2.

TABLE 1

CARBONIC ANHYDRASE ACTIVITY OF TOOTH AND BONE, AND EFFECT ON IT OF ACETAZOLAMIDE, FLUORIDE, AND PARATHYROID

	Concentration	K/Gm	No. Expts.
Human teeth		0.32±0.11	6
Incisors of rats	Normal	3.61±0.33	11
	Acetazolamide 2×10^{-3}	0*	4
	Acetazolamide 2×10^{-4}	1.26±0.35*	6
	Acetazolamide 2×10^{-5}	2.16±0.29	6
	Acetazolamide subcut. 10×10 mg-kg	0.92*	2
	Sodium floride 2×10^{-3}	0*	4
	Sodium floride 2×10^{-4}	1.29±0.36	7
	Sodium floride 2×10^{-5}	2.62±0.44	8
	Parathyroid extract i.m. 10×50 u.	2.99±0.67	2
	Parathyroidectomy	1.41±0.24	7
Femurs of rats	Normal	4.34±0.88	5
	Parathyroidectomy	5.12±0.66	5

* $P < 0.05$.

TABLE 2

ALKALINE PHOSPHATASE ACTIVITY OF TOOTH AND BONE, AND EFFECT ON IT OF ACETAZOLAMIDE, FLUORIDE, AND PARATHYROID

	Concentration	K/Gm	No. Expts.
Human teeth		1.5 ±0.2	7
Incisors of rats	Normal	14.25±1.9	17
	Acetazolamide 2×10^{-4}	5.0 ±1.35*	4
	Sodium fluoride 2×10^{-4}	7.5 ±3.95	4
	Parathyroid extract i.m. 10×50 u.	19.15±1.1*	3
	Parathyroidectomy	10.65±3.2	6
Femurs of rats	Normal	20.35±1.4	4
	Parathyroid extract i.m. 10×50 u.	21.5 ±2.0	3
	Parathyroidectomy	4.0 ±0.8*	5

* See footnote to table 1.

DISCUSSION

As Table 1 indicates, the dental and bone tissues apparently have CAH activity; heating at 90° C. for ½ hour abolished the activity. The fact that the activity was markedly depressed by acetazolamide, a potent CAH inhibitor, at such a low concentration as 2×10^{-5} serves as additional evidence. Mitchell and Johnson[7] found that hamsters, fed diets containing 0.015–0.025 per cent acetazolamide for 3 months, developed three times more dental caries than did the control group. However, at a higher concentration (2×10^{-4}), this substance depressed the APH activity of the same tissue, so that it could not be said to inhibit CAH specifically. As for CAH activity in bone, Siegmund, Dulce, Körber, and Schütte[8] recently reported independently the presence of CAH in the epiphysis of the fowl's bone, using an older colorimetric method, but its absence in the non-ossifying cartilage of the intervertebral disk. In teeth we located the enzyme in the continuously growing incisor of the rat but found practically no activity in the permanent teeth of the adult human.

On the role of APH in ossification, there are a number of hypotheses. For example, Robinson, Macleod, and Rosenheim[3] indicated that the epiphyseal slice from the young rachitic rat was calcified more intensely when incubated in a solution containing a phosphate ester and calcium than in one which contained inorganic phosphate and calcium, which led them to conclude that calcification took place by means of phosphate liberated from the ester through APH activity. In embryonic teeth, the APH level is highest in the dental pulp making the base of odontoblasts,[9] and it becomes suddenly low in the region a little distant.[10] In view of our results, CAH may also be involved in the calcification of dental and bone tissues, and the parathyroid hormone may exert a definite influence on both enzymes in the tissues.

Hyperfunction of the parathyroid gland or administration of the gland extract causes hypercalcemia, hypophosphatemia, increase in APH activity in the plasma and the kidney,[11] and decrease in bone.[12] Conversely, its hypofunction causes hypocalcemia and hyperphosphatemia. The parathyroid hormone has the property of mobilizing calcium of the bone and of producing phosphaturia, simultaneously. Talmage, Toft, and Buchanan,[13] however, assumed from their experiments with radioactive P and Ca that the hormone brings about a mobilization from the bone tissue, as well as renal excretion of both Ca and P. In our experiments, parathyroidectomy caused a significant decrease in APH activity in the bone and a similar tendency, although not statistically significant, in the incisors. The administration of the gland extract increased the activity noticeably. The CAH activity was similarly, but more intensely, influenced by parathyroidectomy. These facts suggest that at least a part of the action of the parathyroid hormone on the hard tissues may be due to its effect on the enzymes concerned with the precipitation or mobilization of calcium phosphate and carbonate.

Fluoride, important clinically as a caries-preventive agent as well as toxicologically because higher doses cause dental damage, is an inhibitor of a number of enzymes. Ichikawa[14] observed a depression or disappearance of APH activity in the pulp and periodontal membrane of the rat fed with sodium fluoride. In our experiments, fluoride was about equipotent with acetazolamide as a depressant of dental CAH, but fluoride inhibited CAH more intensely than APH in the teeth. The deleterious effect of higher doses of fluoride on the teeth may be due to the inhibition of these enzymes.

SUMMARY

Carbonic anhydrase activity was demonstrated by manometric measurement in the incisors as well as in the femurs of rats, but not in human permanent teeth.

Acetazolamide and sodium fluoride were about equipotent as inhibitors of dental carbonic anhydrase. They depressed dental carbonic anhydrase more intensely than alkaline phosphatase (determined spectrophotometrically). Parathyroidectomy caused a significant decrease in alkaline phosphatase activity in the bone and showed a similar tendency, though statistically not significant, in the incisors. The administration of parathyroid extract increased the activity noticeably.

The possible role played by both enzymes under the influence of the parathyroid gland in the calcification of the dental and bone tissues was discussed.

We are greatly indebted to Dr. H. J. Stutz, Lederle Laboratories of the American Cyanamide Co., for generous samples of Diamox.

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The Effects of Some Chemicals on Cervix Dentis

MAMORU YAMADA and SANYA SAKADA

Department of Physiology, Tottori University School of Medicine, Yonago, Tottori, Japan

It is a matter of common observation and experience that the tooth has a rich sensory endowment. The best controlled studies on this subject have been largely histologic. According to Powers,[1] the older reports concerned with sensation in dentin fall into three groups: some which suggest that the dentin is non-innervated,[2, 3] others that indicate the existence of a few nerve fibers,[4–7] and still others that state that dentin is more richly innervated than the finger tips.[8–11] This problem is not solved as yet.[1, 12]

This subject is best studied by an electrophysiologic method which registers the action currents in the dental nerve. Such a physiologic approach was used by Pfaffmann;[13, 14] Yamada, Sugaya, and Hara;[15] Brookhart, Livingston, and Haugen;[16] and Ness.[17] Their investigations clarified the excitation mechanisms of the gingiva, the peridontal membrane, and the pulp that respond to mechanical, electrical, and noxious stimulations. My earlier work suggested that the nerve fiber does not terminate in the dentinal tubule. In this earlier experiment the exciting action of some chemical agents which usually cause hypersensitivity of the dentin was not investigated.

In the present study an attempt was made to find a valuable and fundamental means of acquiring information concerning the etiology of the sensitivity of dentin by investigating the relationships between the effects of chemicals and the modes of actions on the normal cervix dentis, junction of enamel and cement.

MATERIALS AND METHODS

Twenty-six normal dogs, weighing 8–12 kg., were used. A dose of 5–7:5 ml. of cyclopan natrium (*N*-methylcyclohexenyl-methyl-barbituric-Na), administered intramuscularly in 10 per cent aqueous solution was the narcotic. The dosage of narcotic was the minimal dose necessary to produce narcosis in the animals.

After narcosis, the dog's head and lower jaw were fastened to the operating table. The mandibular bone was exposed by removing the skin, and the dental branches of the mandibular nerve, passing through the alveolar canal of the mandible, were stripped as shown in Figure 1. During this procedure, precaution was taken to avoid bleeding. In Figure 1 the solid line of N. alveolaris mandibularis and the branches of the nerve indicate the parts of the mandibular bone exposed. Usually the alveolar nerve divides into three branches: the rami gingivales that innervate the gingival region, and the rami dentales. The latter further divides into two nerves: one of these innervates the periodontal membrane (periodontal branch), and the other, the pulp of the tooth

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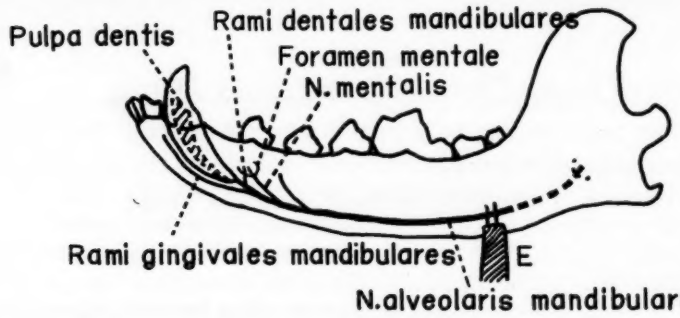

FIG. 1.—Schematic presentation of innervation of alveolar nerves. The solid line of nerve indicates the parts exposed from alveolar canal of mandibular bone. *E* represents the introducing electrodes.

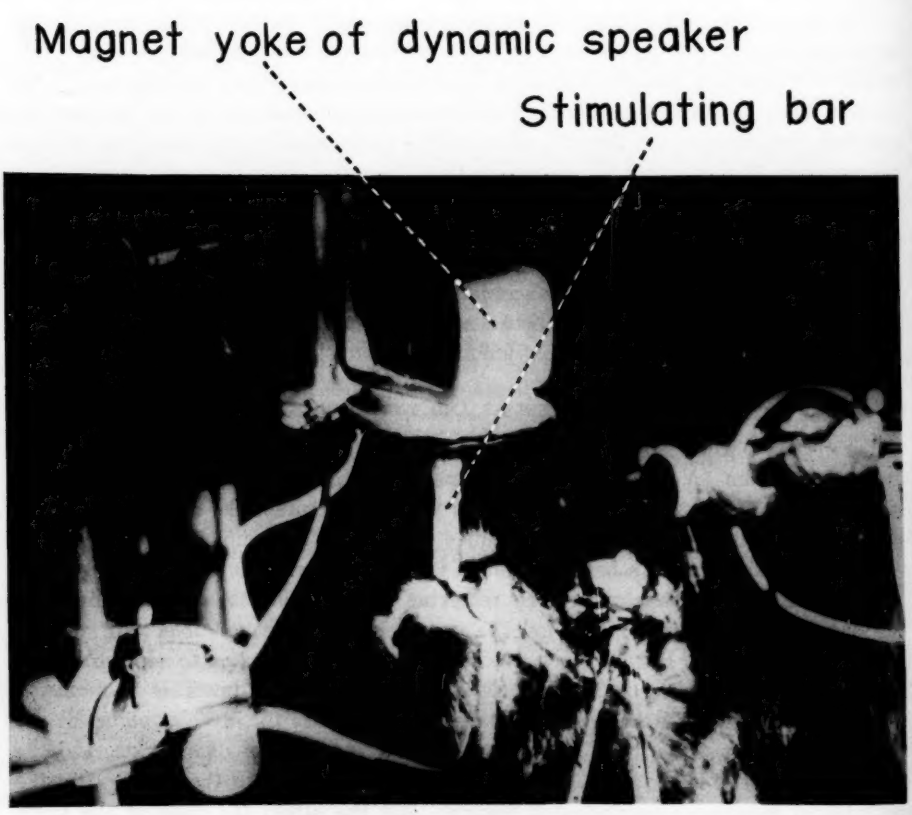

FIG. 2.—The arrangements of the mechanical stimulator

(pulp branch). The distance from the bifurcation of the two branches of the rami dentales to the apex of the root was always 1–1.5 cm. Therefore, it was not difficult to separate and dissect the pulp branch from the other branch. Single nerve fibers were isolated from the pulp branch, and the other nerves were cut. An AgCl type of unipolar electrode introducing the afferent action currents evoked in the pulp nerve by stimuli was settled on the proximal side of the alveolar nerve trunk, as indicated by *E* of Figure 1. The room temperature was kept at 30°–35° C., and precaution was taken to avoid desiccation of the nerve.

Electrical stimuli consisted of single rectangular pulses of controllable parameters, synchronized with the sweep circuit of the oscillograph. Mechanical stimuli were applied to the tooth with equipment that consisted of the voice coil of a dynamic speaker, including a magnetic yoke, and a stimulation by this apparatus. Solutions of varying concentrations of glucose, saccharose, NaCl, NaOH, and HCl were used. These solutions were warmed to 30°–35° C. The cervix of the tooth was exposed by

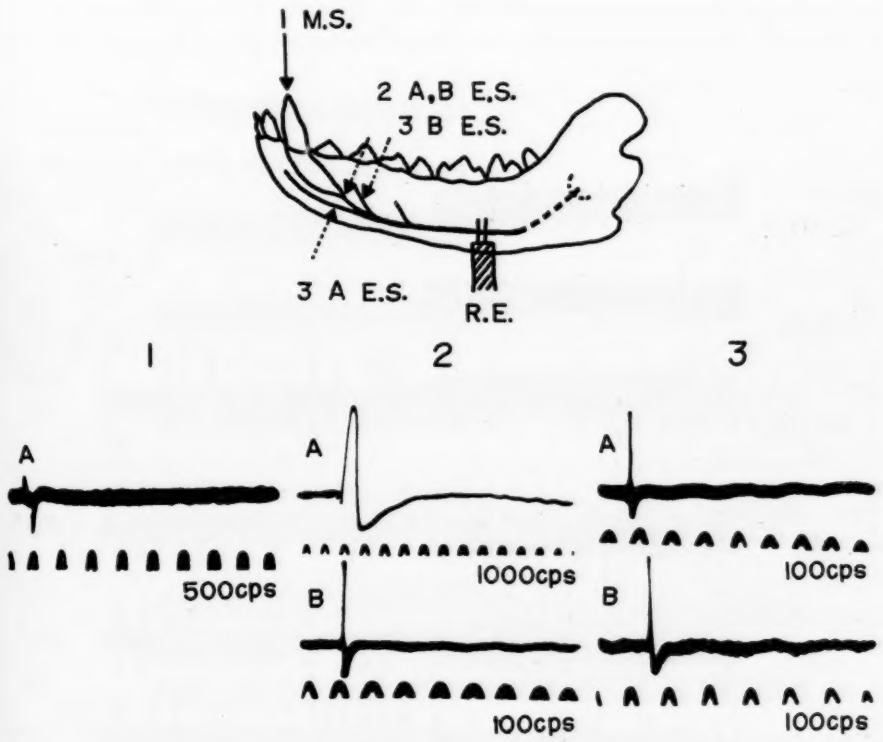

FIG. 3.—*Upper figure:* Diagrammatic representation of the experimental procedures. *M.S.* = mechanical stimulation; *E.S.* = electrical stimulation; *R.E.* = recording electodes; numbers *1, 2,* and *3* and letters *A* and *B* in upper drawing correspond to numbers and letters on tracings below. These tracings were obtained under the conditions shown in the upper figure. The tracings in columns *1* and *2* were obtained from the pulp branch nerve after mechanical stimulation in column *1* and after electrical stimulation in column *2A* and *B*. Tracing *2A* was made in a rapid sweep and *2B* in slow. In column *3*, tracing *A* was obtained after electrical stimulation of N. mentalis and tracing *B* from gingiva branch nerve under the same conditions as column 2.

scratching the alveolar bone, and a piece of cotton wool soaked with the solution under study was applied to it. For the experiment on adaptation of the pulp nerve, the Loewenstein and Rathkampf equipment[18] was used.

RESULTS

Physiologic characteristics of pulp nerve fibers.—In the course of the extended experimental investigation of the nervous mechanisms acting during sensation, it was necessary to investigate the conduction characteristics of the afferent fibers from the tooth pulp described by Brookhart *et al.*[16] Bernick's study[7] has shown that the afferent fibers in the tooth pulp terminate as small, unmyelinated fibers lying among the odontoblasts and without any organized or differentiated structures which might be interpreted as special receptors. His study suggested, too, that, after the terminal arborizations join, the nerve fiber is invested with a myelin sheath. It is reasonable to presume, therefore, that conduction velocities differ according to the condition of the nerve fiber, even in the same nerve fiber. To avoid this discrepancy in this study, the conduction velocity was measured when the pulp nerve was amputated at the apex of the tooth root, and this portion was stimulated by the electrical pulses. The con-

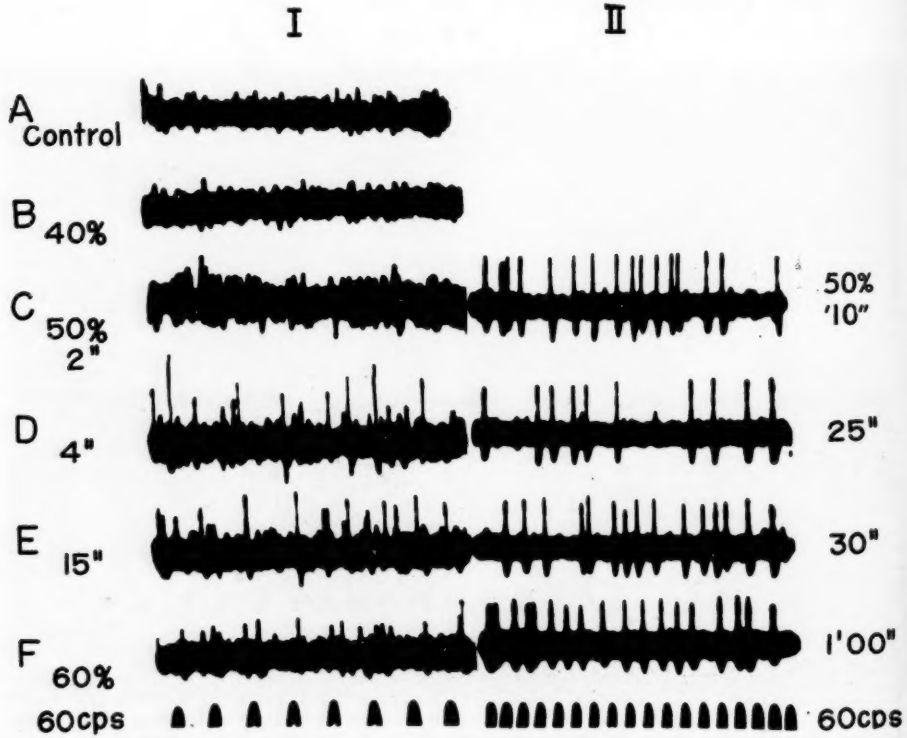

FIG. 4.—Results obtained when a saccharose solution was applied as the stimulus. The results in column *I* were obtained from the nerve trunk of the pulp branch and those of column *II* from a single unit in pulp branch of the other experimental animal. The time values on both sides indicate the lapse of time after stimulation. Lapses of time after stimulus are indicated.

duction velocities were calculated from the difference in time of the different portions of introducing electrodes. In Figure 1, *E* indicates the electrodes to register the action current evoked by stimuli. Figure 3 shows one of the results obtained. In this figure tracing *A* in column 1, which was made from the pulp branch nerve after a mechanical stimulus, is the control. No response was made to the stimulus, and only an artifact of stimulus can be seen. The results in Figure 2, *A* and *B*, were obtained after electrical stimulation. Curve 2, *A*, was obtained when the sweep of oscillograph was rapid and 2, *B*, when it was slow. In column 3, tracing *A* was obtained from the N. mentalis under the same conditions as column 2, and tracing *B* was taken from the gingival branch nerve. Tracings in column 2 indicate that the nerve has normal conductivity. Column 1 does not indicate any loss of conductivity, but it does suggest absence of response to mechanical stimuli and that the conduction velocity of the pulp nerve belongs to the same group of nerve fibers.

The conduction velocities of the alveolar nerves, including the pulp nerve, was

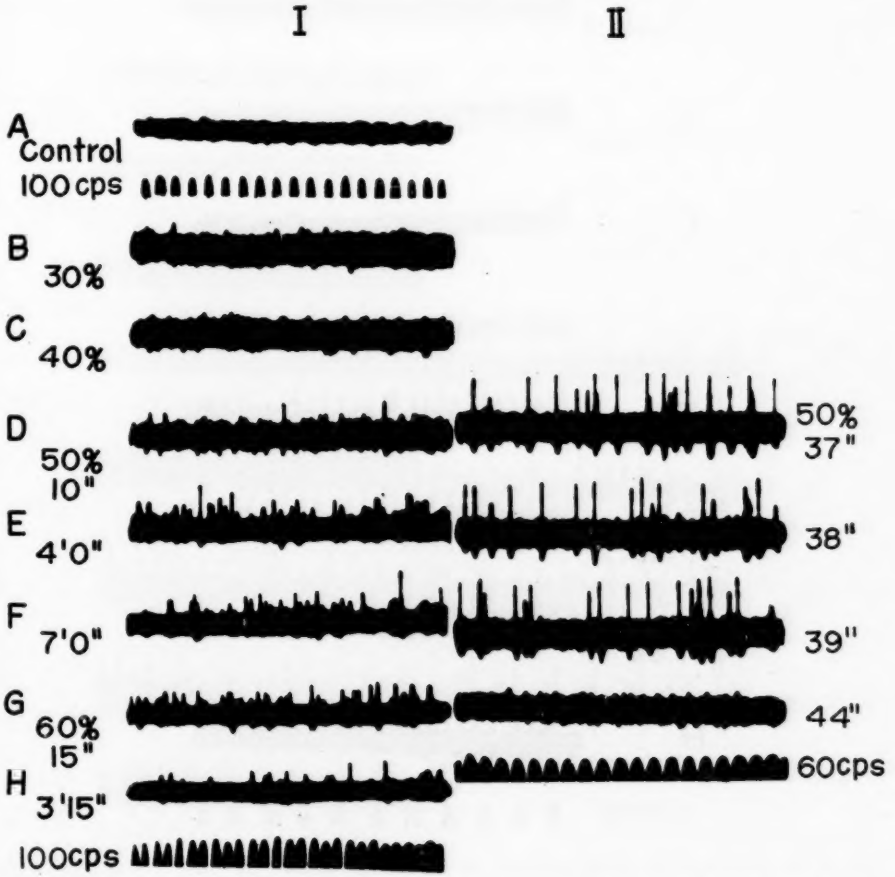

FIG. 5.—Results obtained when the stimulus was a solution of glucose. The details are the same as in Fig. 4.

40–50 m. per second. This is in agreement with the results of Brookhart and associates;[16] that is, this nerve belongs to the A fiber.

The effects of saccharose and glucose on the cervical area.—In the first experiment with chemicals the effects of saccharose and glucose on the pulp nerve were examined. Figures 4 and 5 show these effects. In these figures the tracings labeled series *I* were derived from the trunk of the pulp nerve and those of series *II* from a single unit or a single nerve fiber of that nerve.

The control tracings, *IA* in these figures, illustrate the state in which the stimulus had no effect. Tracings were obtained under threshold conditions with 40 per cent solutions of saccharose or 30 per cent solutions of glucose. When 50 per cent solutions of saccharose and glucose were used, responses increased in number with the lapse of time. In series *II* the same tendency can be seen, but the spiking was extinguished after 44 seconds because of the effect of glucose (Fig. 5). Therefore, the threshold for

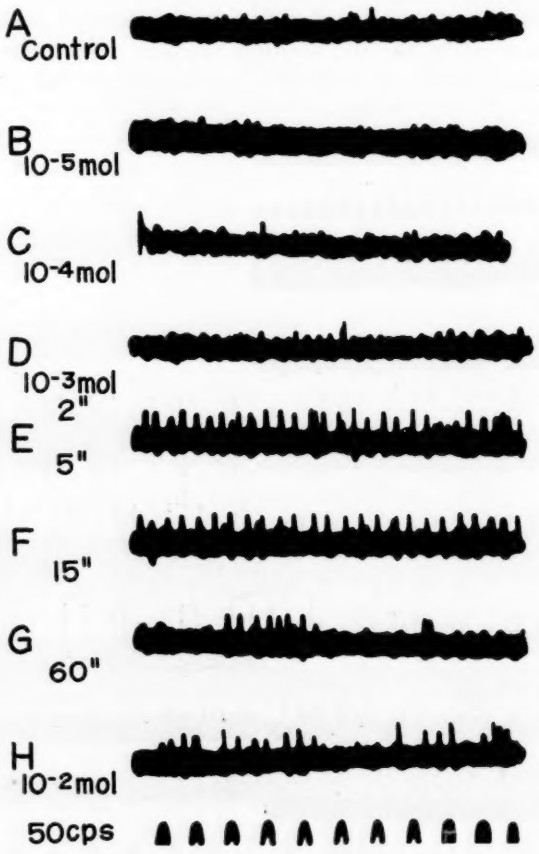

FIG. 6.—Results produced by HCl solution. At the concentration of 10^{-5} mol. (*B*) and 10^{-4} mol. (*C*), no remarkable spikes were shown. On use of 10^{-3} mol. solutions, agitation of the base line was observed in several nerve fibers isolated from the same pulp branch. The volley of discharges was made clear.

concentrations of glucose may be lower than that of saccharose. In solutions at higher concentrations of these substances the frequency and amplitude of spikes decreased with time.

The effects of acid. A solution of HCl was used to determine the effect of an acid on the nerve. Figure 6 presents the results of this experiment. In this figure, *A* is the control; no effect was produced by stimulation of the cervix. Solutions of HCl did not affect the pulp nerve through the dentin at the concentraion of 10–4 mol. When the cervix was immersed in a 10^{-3}-mol. solution of HCl, agitation of the base line was apparent after 2 seconds. Then a few fibers were dissected from the pulp branch

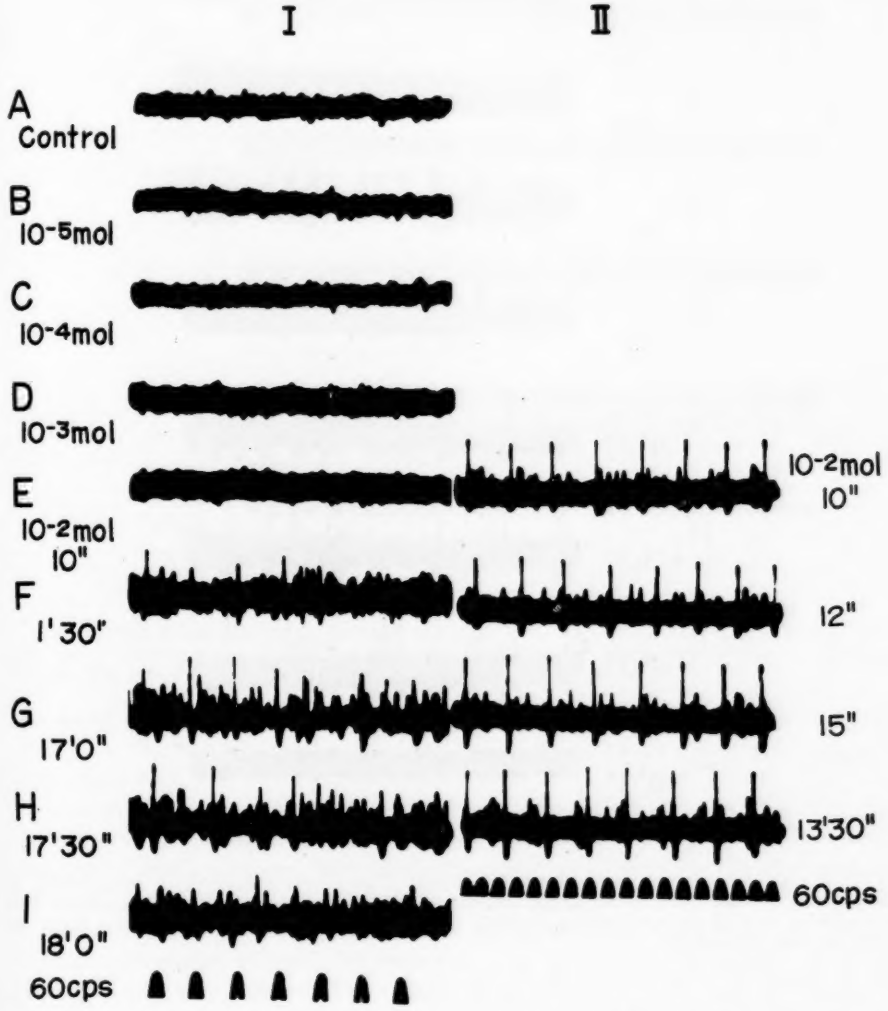

FIG. 7.—Results produced by NaOH solution. The explanation of these results is the same as for Fig. 4.

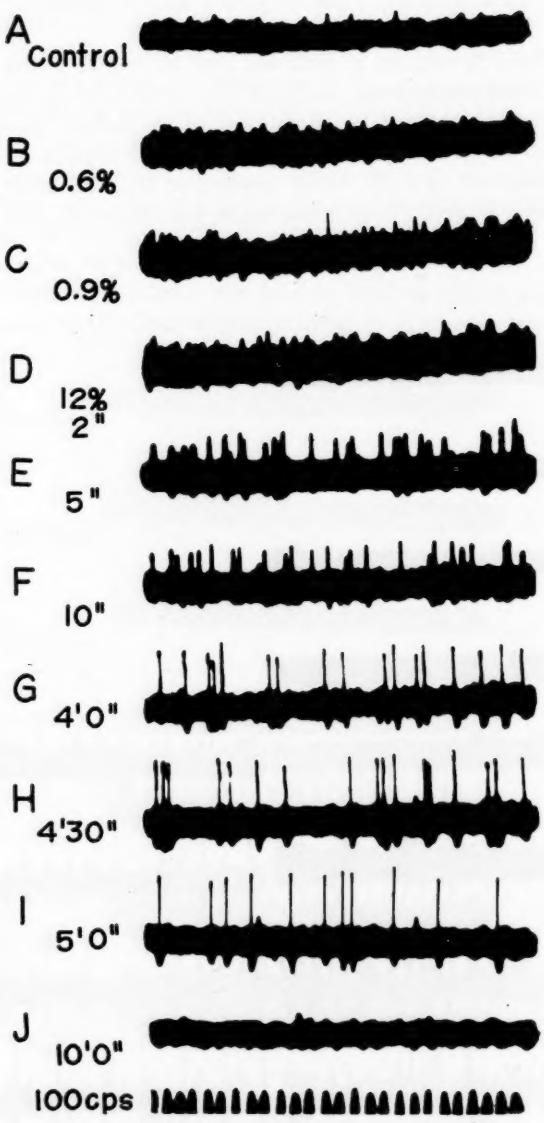

FIG. 8.—Results obtained by NaCl solution. Result *A* shows the control. At a concentration of 0.9 per cent the base line was not stable, and the tendency to spike was made clear 5 seconds after the addition of a 12 per cent solution of NaCl. Tracings *G* to *J* were from a few nerve fibers isolated from the same nerve.

nerve. The repetitive excitations were made clear, as shown in the results *E* and *F* in this figure, but the number of responses decreased gradually 60 seconds after the stimulation began. At a higher concentration, 10^{-2} mol of HCl, the acid solution acted on the pulp nerve through the cervix area, but the responses occurred only at random.

The effects of alkali.—One of the results obtained with sodium hydroxide (NaOH) is represented in Figure 7. The results of series *I* were obtained on the nerve trunk of the pulp branch and those of series *II* on a few nerve fibers. In the control, *IA* of this figure, no changes occurred in the base line on mechanical stimulation. The pulp branch nerve was affected at the concentration of 10^{-2} mol. through the dentin. This

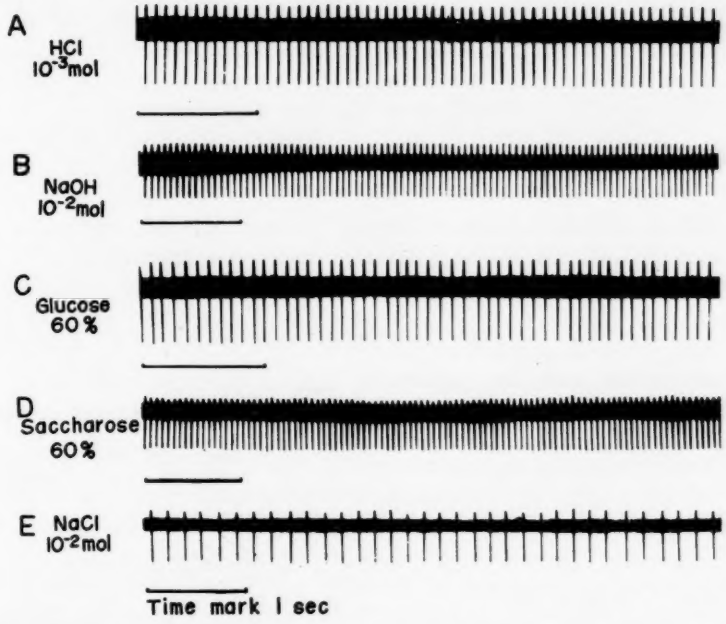

FIG. 9.—Retouched photograph showing excitation of a single nerve fiber from the pulp branch by various chemicals at threshold concentrations. Rhythmic and regular repetitions of responses are shown. The bars under each result show the time marks of 1 second.

appeared obvious when the effects on a few nerve fibers, as shown in series *II*, were observed. The regular, rhythmical, repetitive action currents of the same amplitude are shown.

The effects of salt.—The results of tests with sodium chloride (NaCl) are illustrated in Figure 8. In this figure, result *A* is the control. *C* shows the effect of a 0.9 per cent solution of NaCl; it seems likely from the condition of the base line that excitation of some part of the nerve fibers may be caused by the action of NaCl solution. This is shown more clearly in *D*, *E*, and *F* in this figure when the cervix was soaked in 12 per cent solution of NaCl. To make this result clear, a few nerve fibers of the pulp branch nerve were isolated. The results, *G* to *J* in the figure, were produced. The amplitude of the spikes indicates the same height according to the all-or-none law, but 10 minutes after stimulation the nerve did not show any response.

The relations of the chemicals to the adaptation.—The response to saccharose, HCl, NaOH, and NaCl solutions decreased gradually under the conditions of the threshold concentrations of these chemicals.

In this section the variations of adaptation by these chemicals were investigated. A single nerve fiber was isolated from the pulp nerve, and the potentials evoked by the manipulations were registered and observed for a long period. As the stimulating concentrations of these chemicals, the approximate values of the threshold concentrations were used. Figure 9 illustrates some of the results, and Figure 10 presents these results diagrammatically. Remarkable changes in response intervals are not shown. The relations found on longer observation are shown in Figure 11. In this figure the number of responses in one sweep of 0.6 and of 0.2 second's duration of the cathode-ray oscillograph are shown in an observation time of 1 minute. Based on these findings, the sensory unit, consisting of the odontoblast and nerve terminal, apparently indicates the slow-adapting unit as described by Loewenstein[19] and Skogland,[20] namely, a stationary discharge in response to steadily maintained stimulation by chemicals.

DISCUSSION

Since pain in teeth has a practical meaning in our daily life, the mechanisms by which some chemicals provoke hypersensitive dentin or excite the dental pulp nerve

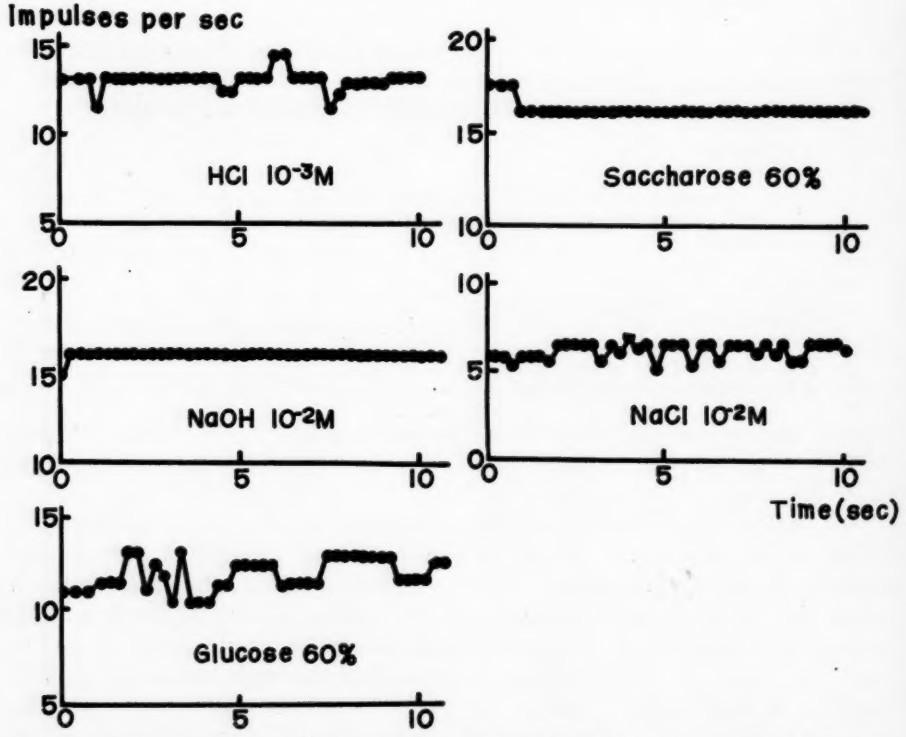

FIG. 10.—Diagrammatic representations of the results obtained in Fig. 9. On ordinates the frequencies per second are shown and on abscissas the periods of stimulation are indicated.

appear worthy of discussion. For the purpose of an extended investigation on these subjects, it has been necessary to determine the characteristics of the afferent nerve fiber of the tooth pulp. Conduction velocities of 40–50 m. per second were obtained. Therefore, the pulp nerve fibers have conduction characteristics that resemble those of the A gamma-delta fibers of the cat. This result is essentially similar to results reported by Brookhart and associates.[16] Histologic investigations have disclosed that nerve fibers were predominantly unmyelinated;[21] Windle's work,[22] however, suggested that there were unmyelinated nerve fibers in the sensory root of the trigeminal nerve.

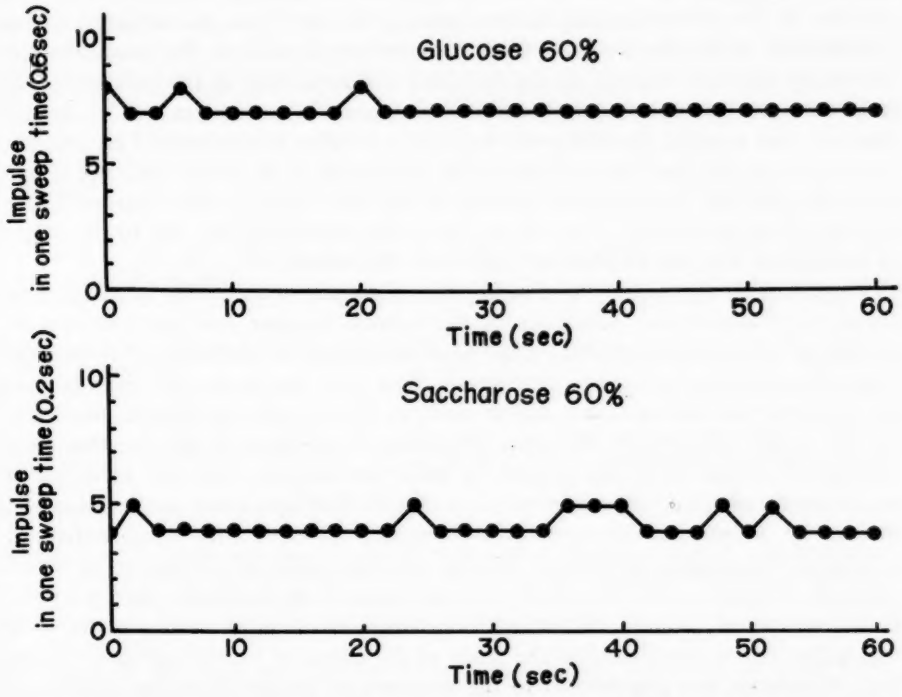

FIG. 11.—Diagrammatic representations of the relationships between the variations of frequencies and the long stimulating periods in glucose and saccharose solutions. On the ordinates the number of responses in one sweep time is shown, and on the abscissas the observation times from the beginning of the stimulation are indicated.

Functional studies of afferent fibers in the pulp branch by Pfaffmann[13, 14] suggested that these fibers belong to the group of fibers with slow and variable conduction velocities. As the results of Bishop and Heinbecker[23] and Brashear[21] showed, the pain-conducting afferent fibers are both myelinated fibers of the smaller diameters in the A group and unmyelinated nerve fibers of the C group. A recent work carried out with an electron microscope by Uchizono and Homma[24] indicated that a great number of the nerve fibers of the tooth pulp were medullated and others were not. Most of the latter were found near, or attached to, the blood vessels; the site suggested their relationships to the autonomic funcion. From the findings of Uchizono and Homma, it was thought that the afferent nerves that we investigated were a group of myelinated

nerve fibers. On the basis of our evidence, it seems likely, too, that only one group of action currents can be induced from the alveolar nerve. Further studies on this problem are necessary, however.

On chemical stimulation of the cervix of the tooth, numerous discharges occurred. The experiments strongly suggest that the pulp nerves are excited when these chemicals are applied to the cervix of the tooth. The conduction mechanisms have not been made entirely clear as yet. There are many theories concerning this problem. Some investigators maintain by histologic observations on the absence of the nerve fiber in the canaliculus that the odontoblasts in the dentinal tubules conduct the stimulus to the nerve terminals. Others support the view that the excitation of the pulp nerve is evoked by the nerve fiber, the existence of which in the canaliculus was proved by histologic staining. If the resistance is a nerve fiber in the canaliculus, the pulp nerve will be excited when the dentin is scraped. My associates and I[15] showed, however, that scraping the dentin did not release a volley of discharge. This evidence strongly favors the view that the excitation mechanism is the dentin and that it conducts the impulses to the nerve terminal of the pulp nerve. Powers' studies[17] have indicated that the finding of the nerve fiber in the canaliculus was due to the failure of the method and was an artificial product of the staining.

Because of the concentrations of solutions of chemicals necessary to cause excitation threshold concentrations, variations of the osmotic pressure and the hydrogen-ion concentration (pH) are regarded a the principal sources of excitation. However, the threshold concentrations were unexpectedly high. The reason for this may be that the chemical solutions could not diffuse easily to odontoblasts or dentinal canaliculus because of the thickness of the tissue. Therefore, it is logical to presume that for a solution to act on the dentin it must be more concentrated than that necessary to reach the threshold of excitability if it is applied directly to the nerve. From this inference it is clear that the responses to the stimuli appeared a few seconds after the stimulatus was applied to the cervix area and the volley of discharges decreased gradually in number when threshold concentrations of the chemicals were employed in our experiment, because the concentrations were too high to excite directly. It is reasonable also to speculate that the grade of the effects of various agents or pathologic stimulation are proportional to the thickness of the cervix or the junction of enamel and cement. Based on these inferences, it is not difficult to account for many clinical symptoms.

As a result of this system of adaptation, the stimulation activated the volley of discharges for a long time. Therefore, this system belongs to the "slow-adapting unit" as described by Loewenstein.[19] However, whether it is the characteristic of the odontoblast or of the nerve terminal attached to the cell body is not clear. The significance of the conduction mechanism of excitation has not been assessed, and this problem presents an intriguing field for research.

SUMMARY

The function of the afferent fibers of the pulp branch of N. alveolaris mandibularis on stimulation by some chemicals was studied in 26 dogs narcotized with *N*-methyl-cyclohexenyl-methol-barbituric sodium in a search for valuable and fundamental information relative to the etiology of hypersensitive dentin. It was found that the

conduction velocity of the afferent nerve resembles that of the A group of cat's nerves.

As stimulating agents, glucose, saccharose, HCl, NaOH, and NaCl were used. Afferent volleys initiated chemically appeared when 50–60 per cent solutions of glucose and saccharose, 10^{-3} mol of HCl, 10^{-2} mol of NaOH, and 12 per cent solutions of NaCl, respectively, were used. Chemical stimulation on the cervix dentis evoked volleys of discharges for a long time. The conductivity and adaptation mechanism of the system, which consisted of odontoblast and nerve terminal, were discussed.

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Effects of Temperature Change on Mandibular Blood Flow

E. EARL MOORE, JACK G. BISHOP, J. LESTER MATTHEWS, and HOMER L. DORMAN

Department of Physiology, Baylor University College of Dentistry, Dallas, Texas

A number of investigators have demonstrated that heat may augment the flow of blood in peripheral areas. Conversely, cold has been routinely utilized to reduce blood flow in peripheral areas. Flax, Miller, and Horvath,[1] using diathermic heating of tissues, reported four types of responses resulting from its application. Richardson, Imig, Feucht, and Hines[2] believed that the response depended on the magnitude of heating and the blood-flow rate prior to heating. Feucht, Richardson, and Hines[3] found that the application of single hot packs resulted in small temperature elevations and caused slight or inconsistent increases in blood flow. However, repeated application of hot packs increased blood flow concomitantly with increase in subcutaneous temperature. Kemp, Paul, and Hines[4] reported that microwaves significantly increased blood flow in the periphery but that results with other heating methods were more variable.

The common use of heat therapy and the paucity of experimental evidence on the mechanism of action of heat and cold in oral tissues suggest the importance of determining the effect that these two therapeutic agents have on blood flow, blood pressure, and peripheral resistance in the mandibular regions.

EXPERIMENTAL METHODS

Blood-flow and blood-pressure measurements were simultaneously recorded in the right mandibular artery of mongrel dogs by means of direct cannulation at the level of the mandibular foramen.[5] The animals were anesthetized by intravenous infusion of sodium pentobarbital, 33 mg/kg. Heparin, 5 mg/kg, was used as an anticoagulant. Blood flow was recorded in milliliters per minute, using an electromagnetic blood flowmeter.[6] Lateral blood pressure was recorded by the use of a strain gauge, strain analyzer and ink-writing oscillograph. Peripheral vascular resistance was calculated by dividing blood flow into blood pressure[7] and was recorded as peripheral resistance units (PRU).

Temperature was intermittently monitored by the use of thermocouples. One thermocouple was inserted into the soft tissue lateral to the mandible, another into the soft tissue mesial to the mandible, a third into the rectum, and the fourth placed in the laboratory to monitor room temperature.

Infrared heat was applied by a 125-volt infrared lamp, at a distance of 10 inches

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from the mandible. Cooling was accomplished by direct application of a latex ice pack to the area lateral to the mandible.

RESULTS

Six experimental animals varied in weight from 33 to 48 pounds. The lateral mandibular temperature averaged 36° C. prior to experimental heating or cooling. Control blood flow varied from a low of 2.2 ml/min to a high of 14.0 ml/min, with an average of 5.5 ml/min. Average blood pressure was 110.7 mm. Hg, varying from a low of 70 to a high of 150 mm. Hg. The control peripheral resistance averaged 30.6 units.

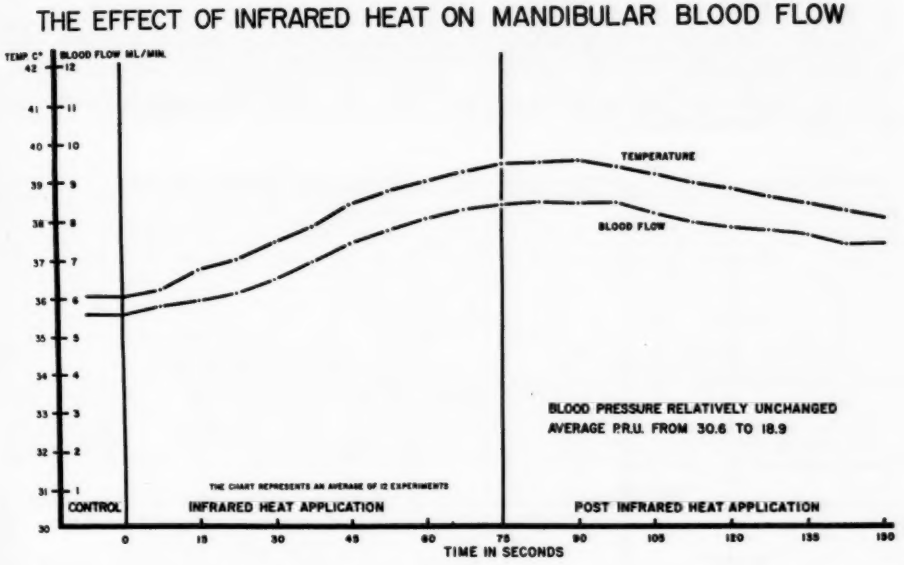

FIG. 1.—A graph showing the average blood flow and temperatures induced by twelve infrared exposures on the mandibular area.

Infrared heating for 1½ minutes increased the lateral temperature an average of 3.6° C. Blood flow increased as much as 158 per cent and as little as 32 per cent, with an average increase of 66 per cent over control value. Arterial blood pressure did not significantly change. Peripheral vascular resistance decreased from 30.6 to 18.9 units. In all experiments, heating resulted in a blood-flow increase and peripheral resistance decrease.

The relationship between blood flow, lateral temperature, and time is shown in Figure 1. Continuous recording of mandibular blood flow permitted an evaluation of the time course of infrared heating and blood-flow changes. The graphic representation of these data presents an average of twelve experimnts on six animals. A typical record is shown in Figure 2.

Eleven experiments, using 7 animals, were conducted with ice-pack cooling. Control blood flow averaged 8.2 ml/min, varying from a low of 0.5 to a high of 20.3 ml/min.

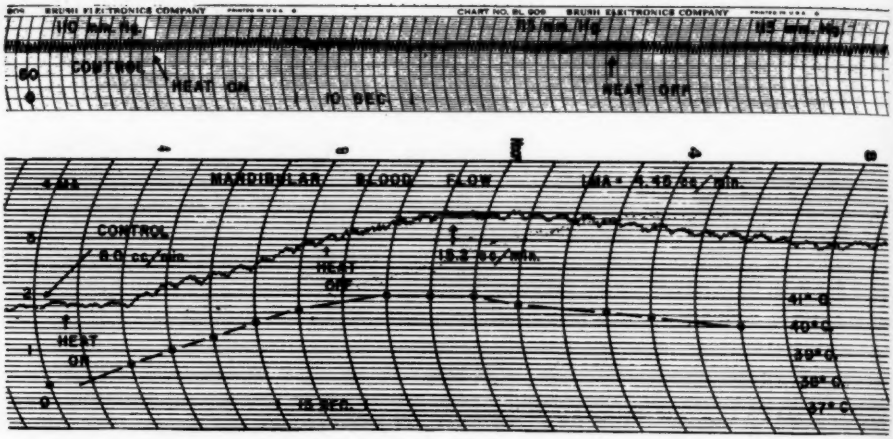

FIG. 2.—Records of mandibular blood flow and blood pressure in response to heat. Temperature changes are shown in dotted line.

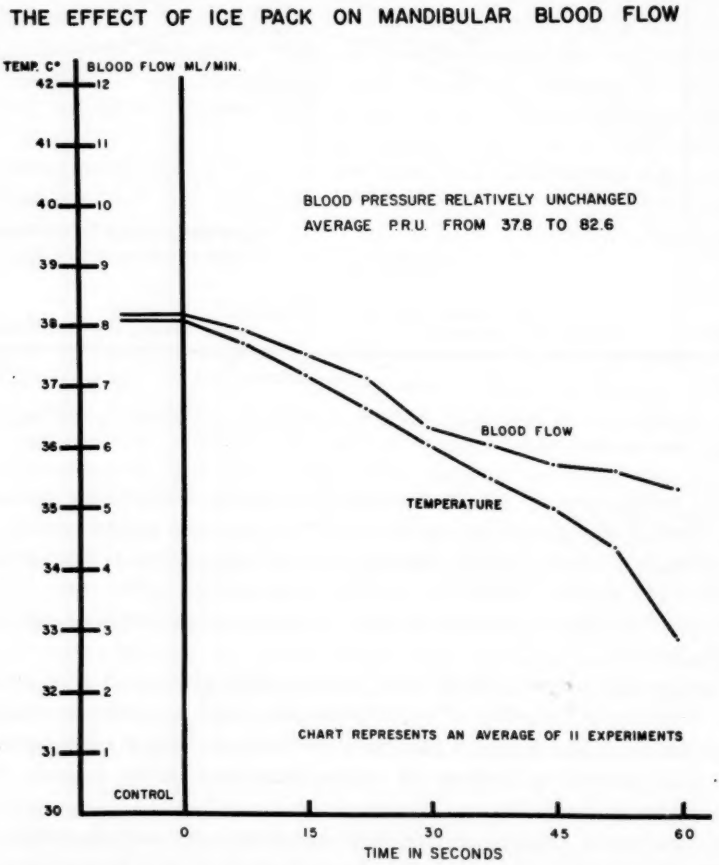

FIG. 3.—A graph showing the average blood flow and temperatures induced by eleven ice-pack exposures on the mandibular area.

Control arterial blood pressure ranged from 70 to 145 mm. Hg, with an average of 100 mm. Hg. The average resistance (PRU) was 37.8.

Following the application of ice packs, blood flow decreased an average of 47 per cent, varying from a low of 9 per cent to a high of 100 per cent. Arterial blood pressure did not change significantly. Peripheral vascular resistance increased from a low of 8 per cent to a high of 300 per cent, with an average of 96.6 per cent increase.

In all experiments, cooling resulted in decreased blood flow and increased peripheral resistance. The relationship between blood flow, lateral tmperature decrease, and time is shown in Figure 3. Figure 4 is a typical recording obtained from these experiments.

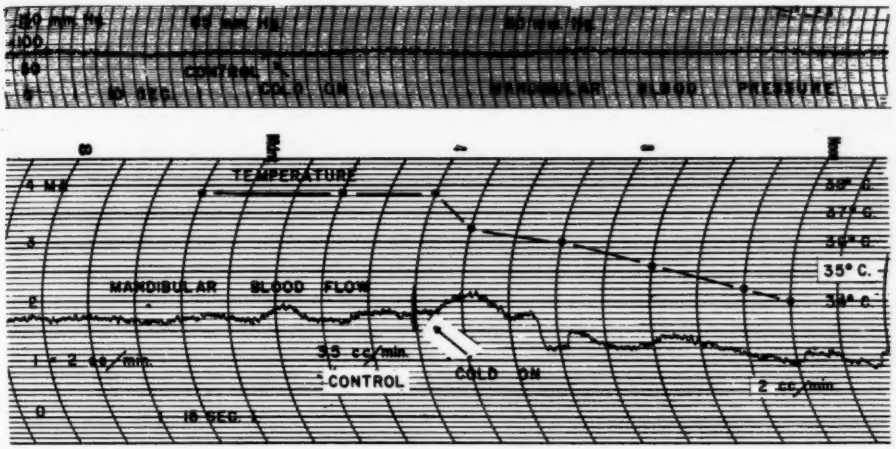

FIG. 4.—The effect of cooling on mandibular blood flow and blood pressure. Temperature changes are shown on blood-flow record.

DISCUSSION

Richardson,[8] using microwave–induced heating on skeletal muscle, found increases in blood flow up to 85.7 per cent above control levels. It was also reported that there was a critical temperature, 44.1° C., at which blood flow began to increase significantly in skeletal muscle. A critical temperature phenomenon was not demonstrated in this research. On the contrary, blood-flow increase began as soon as the tissue temperature began to rise. Further temperature increases continued to augment blood flow.

Kerslake[9] found that the increase in blood flow following heat was not prevented by cutting off the circulation and believed that the response was due to direct stimulation of afferent nerve endings. It has been shown that the vascular response to heat depended on the integrity of the vasomotor supply. A striking similarity exists between the blood-flow response to effective doses of acetylcholine and the application of heat.

Effective doses of epinephrine have been shown[5] to constrict the vascular bed of the tissues supplied by the mandibular artery. Acetylcholine, on the other hand, is a vasodilator to the blood vessels of the same area. This investigation has shown that

the application of cold results in a tissue response resembling that produced by epinephrine and that heating these tissues yields a response similar to that induced by acetylcholine. The simple correlation between these two phenomena does not necessarily imply that the same physiologic mechanisms are involved.

Since peripheral resistance was the factor most affected by the temperature change, it may be assumed that the mechanism is either a local reflex or a direct effect of temperature change on the arteriole. Body temperature did not significantly change during the series; therefore, no central nervous system of temperature regulation appears to have been involved.

SUMMARY

The effects of infrared heating and ice-pack cooling on mandibular hemodynamics have been determined by the use of an electromagnetic blood flowmeter and simultaneously recorded arterial blood pressure. Infrared exposure for 1.5 minutes increased blood flow and decreased peripheral resistance. Blood pressure was not significantly changed.

Application of ice packs for 1 minute decreased blood flow and increased peripheral resistance. Arterial blood pressure remained largely unaltered.

Since the peripheral resistance changes resulted primarily from blood-flow variations, the vasomotor mechanisms were considered to be mediated by either a local reflex or the direct effect of temperature change on the vascular bed.

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Oral Disease in Yeshiva Students

KURT A. ROSENZWEIG and ANSELM LANGER

Department of Preventive Dentistry, Hebrew University and Hadassah School of Dentistry, Founded by the Alpha-Omega Fraternity, Jerusalem and Malben-JDC, Tel-Aviv

Epidemiologic surveys may serve various purposes.[1] Sometimes they are conducted for scientific reasons, to investigate the incidence of certain illnesses in a population in order to try to establish a causal connection between some known characteristics of this population and its specific pattern of morbidity as compared with another population which does not possess these characteristics. Also, data on morbidity are collected to serve as a basis for planning or for evaluating the effectiveness of health programs in this population.

The main purpose of the present investigation was to provide data on the needs in the field of oral health of Yeshiva (Talmud academy) students, for whom medical and dental care is provided by Mif'al Hathorah, an organization whose medical and dental activities are supervised by Malben; Malben represents the services of the American Joint Distribution Committee in Israel. In order to assess the scope of the needs of the Yeshiva students, the medical department of Malben decided to carry out a comprehensive health survey on members of this community in Jerusalem. As part of this survey, the dental division, in conjunction with Mif'al Hathorah, organized an investigation of oral morbidity.

Since data in this field had been previously collected in various parts of the population, it appeared to be of interest to compare a specific and well-determined group as the Yeshiva students with the general population. For this reason, the School of Dentistry of the Hebrew University in Jerusalem decided to take part in the study.

MATERIALS AND METHODS

Most of the Yeshiva students are of Eastern European origin. The greater part belong to families which have been living in the country for many generations. Only a small minority immigrated recently from the same region as well as from North Africa and the Yemen.

These people observe strictly all the laws prescribed by the Jewish religion, including dietary regulations. They live in quarters separated from the general population. Very seldom do they visit regular schools. Most of them devote themselves from childhood exclusively to the study of the Holy Scripts and the Talmud in special schools, the Yeshivoth. Here they learn, besides writing and reading (in Hebrew), elementary arithmetic and a few additional subjects of basic knowledge. But, in spite of their seclusion, they take a keen interest in all aspects of life and not the least in politics. Only exceptionally do they learn and perform a trade to gain a living. As

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a rule they spend the greatest part of their life in the Yeshiva, studying the Holy Books, teaching, learning, and discussing the inexhaustible problems of the Talmud. They derive their livelihood mainly from money which is collected in Israel and abroad, in order to enable them to abstain from worldly occupation and devote themselves to their studies and prayers. Thus it is understandable that economically they belong to the lower classes. As a rule, they have large families and live in crowded quarters.

A random sample of 414 people was selected. These comprised about 15 per cent of the Yeshiva students of Jerusalem and were representative of the whole community. The sample included students from five to fifty years of age and above, who were divided into groups of 5 years. The findings were analyzed with regard to age and continent of origin (according to birth of the father).

Examinations were carried out by two previously calibrated dentists in the dental clinic of Mif'al Hathorah, in daylight. They used spotlights, plane mouth mirrors,

TABLE 1

INCIDENCE OF CARIES BY AGE

Age (Years)	Examined	DMFT Average	Percentage of at Least 1 DMF Tooth
5– 9	39	0.84	35.9
10–14	94	3.41	79.8
15–19	157	3.92	84.1
20–24	70	5.13	91.43
25–29	28	7.28	100.0
30–34	11	7.90	100.0
35–39	7	8.57	100.0
40–44	3	9.66	100.0
45–49	2	11.00	100.0
50 plus	3	11.00	100.0

explorers, and compressed air. One dentist examined for caries and gingivitis, and the other for periodontal disease and malocclusion. The examiners themselves entered their findings in specially designed forms on which demographic details about the examinee had been recorded.

Accepted epidemiological indices were used: for caries, the DMFT count;[2] for gingivitis, the P-M-Aa index;[3] for periodontal disease, Russell's score (PI);[4] and for malocclusion, the HLD index.[5] The records were then preliminarily summarized in the statistical department of Malben and the findings transferred to punched cards and processed.

RESULTS

Table 1 shows the prevalence of caries of the sample by age, ranging from 0 to 20 DMF teeth. The number of teeth affected per individual rose with age in a straight-line fashion up to the age group twenty-five to twenty-nine. The percentage of individuals having at least one DMF tooth followed the same pattern. After this age the average DMF rose at a considerably lower rate, and the percentage of people affected reached the maximum.

Table 2 gives a further breakdown of caries experience by age and origin. Since in some of the groups there was not a sufficient number of students to warrant a comparison, only the age groups from ten through twenty-four years were included. It was found that caries was highest in people born in Israel and that it decreased in the order of Europeans, Africans, and Asians, but differences between these groups were statistically not significant.

The distribution of gingivitis by age and origin is given in Table 2. There were no statistically significant differences between any of the groups of origin, but prevalence of disease was higher in Europeans than in the other groups. This fact became even more pronounced when the weighted P-M-A[6] for assessment of the total amount of gingivitis was applied. In all groups of origin, "P" reached a peak between the years ten and fourteen, and the relative importance of the factor decreased gradually. In

TABLE 2

INCIDENCE OF CARIES, GINGIVITIS, PERIODONTAL DISEASE, AND MALOCCLUSION BY ORIGIN AND AGE

Origin	Age (Years)	No. Examined	DMF	P	M	P-M-A	PI	HLD
Israel	10–14	77	3.69	5.32	2.70	10.68	0.15	.....
	15–19	106	4.42	4.60	3.39	12.31	0.14	.....
	20–24	53	5.57	3.94	4.79	16.45	0.24	.....
Europe	10–14	8	2.13	3.75	3.38	11.04	0.19	.....
	15–19	24	4.17	4.91	6.83	16.73	0.26	.....
	20–24	11	3.90	4.45	5.27	23.71	0.66	.....
Asia	10–14	7	2.57	4.71	2.43	9.29	0.20	.....
	15–19	15	1.67	4.67	3.40	12.73	0.19	.....
Africa	15–19	12	2.67	6.92	3.83	13.92	0.20	.....
Israel	5–17	221	.....	.....	.....	.....	.....	2.71
Europe	5–17	32	.....	.....	.....	.....	.....	3.94
Asia	5–17	23	.....	.....	.....	.....	.....	1.65
Africa	5– 7	14	.....	.....	.....	.....	.....	3.35

contrast, a slow rise in "M" was recognizable, and it surpassed "P" between twenty and twenty-four years. It is for this reason that the total P-M-A rose with age. Since inflammation of the attached gingiva was only exceptionally seen, this factor was not specially analyzed.

A similar pattern was observed in periodontal disease, as assessed by Russell's index. Again, Europeans were more affected in all age groups, but no statistical significance of the difference was found.

Severe malocclusion, as expressed in terms of the HLD index, was most prevalent in Europeans, who were followed by Israelis, Asians, and Africans, in that order.

In order to establish whether there existed a connection between any two of the conditions investigated in the present study, indices were calculated for the whole sample, and then the correlation was estimated in terms of a correlation coefficient. A high correlation was found between the weighted P-M-A ($r = +0.798$) and Russell's index; one of medium grade between P-M-A and HLD ($r = +0.599$). Both correlations were significant. Between the rest of the indices no significant correlations could be established.

DISCUSSION

Two studies on caries have been published so far in Israel which may be compared with the present findings. Weinreb and Ben-Shushan[7] reported caries experience of young men from eighteen through thirty years in the army. The values of the average DMF which were found by these investigators are shown in Figure 1, together with those of the present studies, for which the regression line has been calculated by the method of least squares. It is evident that findings in both studies are practically identical with regard to caries prevalence as well as regarding caries incidence as expressed by the slope of the line. In another study[8] it was found that caries prevalence in children thirteen to fourteen years of age was between 2 and 3 DMF teeth in Israel, varying with the origin of the children. The group which corresponded ethnically

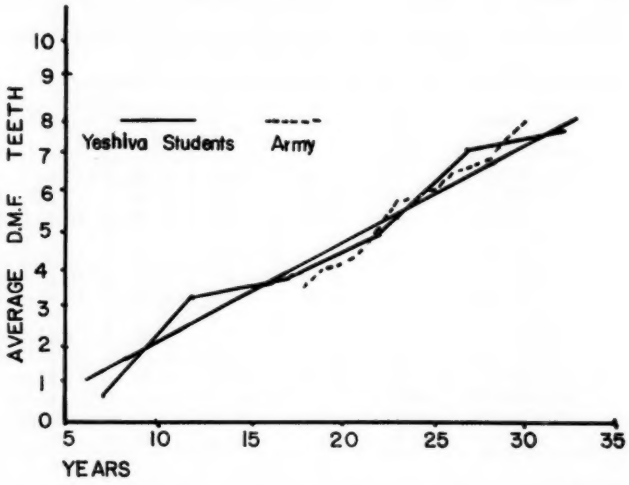

FIG. 1.—Values of average DMF in army men aged eighteen to thirty years compared with Yeshiva students.

most closely to the majority of the Yeshiva students showed the higher value. The average DMF actually found in the present investigation by interpolation on the regression line was 3.05 DMF teeth. Thus it is shown by three independent studies giving identical results that caries incidence is low in Israel in comparison with other civilized countries.

The afore-mentioned investigation showed a significantly higher caries experience in children of Western origin (including Israelis of second generation) than in oriental children (Yemen, North Africa, Iraq-Iran, Arabs). The same pattern was found in the present survey, although no statistically significant difference could be established.

The prevalence of gingivitis has also been investigated by means of a sample survey of school children thirteen to fourteen years of age. Results have been published elsewhere in detail.[9] Although the age groups in the present study were different, it appears permissible to compare group ten to fourteen years to the former findings. It has been established by various investigators that there is a progressively increasing

P-M-A score observable in this period of life with increasing age, so that inclusion of younger children with thirteen- to fourteen-year-olds should, on the whole, lower the average P-M-A. Nevertheless, gingivitis was found to exist at a much higher rate in Yeshiva students than in the general child population of the same origin and was of about the same order as in North Africans, who were found to have significantly more gingivitis than other Jewish communities. It has been repeatedly reported[10–13] and also shown in a recent study on a limited scale carried out in Israel[9] that gingivitis is most closely correlated with the status of oral hygiene. Although this factor was not objectively assessed in the present investigation, it is generally felt by dentists who are familiar with this group that there is room for much improvement in the oral hygiene of Yeshiva students.

The periodontal index is relatively new and has been applied in the present study for the first time in Israel. If findings are compared with the data found by Russell *et al.*[2, 14–15] in different studies, it appears that, in the lowest age group, the score of the Yeshiva students was higher than in both the American urban and rural populations. Undoubtedly, this fact reflects the relatively high amount of gingivitis in the Yeshiva children, which is also estimated by the PI. Contrarily, the prevalence of periodontal disease was low in the Yeshiva students and of about the same order as in people with the highest educational status in the Birmingham study.[2] Russell also found a positive correlation between periodontal health and the extent to which dental care, as expressed by filling or extraction of carious teeth, had been received.[2] In the present study the DMF of the total population was 4.42, D being 2.93, M = 0.57, and F = 0.92. Thus, the ratio $(M + F)/DMF \times 100$ is only 33.7, or one-third of the needs for extractions or fillings have been met; but it should not be overlooked that these needs were much less extensive in Israel than in the United States because of the lower DMF rate and lower percentage of individuals having at least one DMF tooth. Therefore, it appears that lack of professional dental care has a much less aggravating influence in Israel on the state of the periodontal tissues. Unfortunately, the presence of materia alba as an indicator of oral hygiene was not recorded; thus it is not possible to assess this factor in connection with the PI scores.

The HLD index (Table 2) was chosen for the estimate of malocclusion, since this index was devised primarily for the investigation of needs for treatment. Therefore, only children up to the age of seventeen years were examined as to malocclusion.

As in the survey on oral health of schoolchildren where Angel's classification had been applied,[16] a positive and significant correlation between gingivitis and malocclusion was established.

SUMMARY

A representative sample of Yeshiva students, including 414 individuals between five and fifty years of age, was examined as to caries experience, gingivitis, periodontal disease, and malocclusion. No statistically significant differences with regard to origin were found in any of these conditions. With regard to incidence of caries, this group followed the same pattern as the general population of the country. Gingivitis was much higher than in comparable age groups of the same origin. Periodontal disease was found to the same extent as in comparable groups of high educational status in Birmingham, Alabama.

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Permeability of Investment at the Casting Temperature

JOHN S. SHELL and EDWARD R. DOOTZ*
Ransom & Randolph Co., Toledo, Ohio

It is probably essential for the production of sound dental castings to provide in some way for the elimination of the gas in the mold cavity during the casting procedure. The gas permeability of investments should be adequate to allow the gas to pass through the hot investment at the rate that the metal enters the mold.

Some measurements of gas permeability have been made, but these tests have been performed on cold investment after the mold had been burned out and cooled to room temperature. Such tests do not provide data pertinent to the actual conditions present when a casting is made. Investments shrink when cooled to room temperature after being burned out, and it is highly probable that this has a decided effect on the permeability. Numerous cracks may form during the cooling and may drastically affect the permeability. It is also possible that the shrinkage causes an increase in density of the investment which might also affect the permeability.

EXPERIMENTAL METHODS

The permeability of investments at the casting temperature employing pressures in the range of those encountered in dental casting was measured. The equipment is illustrated in Figure 1. Dry air at a slight pressure (5 cm. Hg) was passed through the monel tube and the investment sample during the burn-out to prevent water and wax vapor from collecting in the cold end of the monel tube. The readings were made by passing air, at a controlled pressure, through the train, which includes the flowrator, a monel tube with adapter (Fig. 2), and the investment sample in the furnace. The investment and the adapter (Fig. 3), were heated in the furnace employing any desired burn-out cycle. The readings were made at the time and temperature at which the casting would normally be made. The compressed air was dried by first passing through a desiccator containing an anhydrous calcium sulfate.† The pressure was held constant by a pressure regulator and was indicated by the mercury manometer. The flow of air was measured by the flowrator‡ (Fig. 4), containing a floating ball in a tapered glass tube.

The flowrator was calibrated by removing the investment specimen and substituting a needle valve. The valve was adjusted to give desired readings on the flowrator, and the gas was collected in a burette. The flow of gas in cubic centimeters per minute at

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* Present address: J. F. Jelenko Co., New York, N.Y.

† Drierite, from W. A. Hammond Drierite Co., Zenia, Ohio.

‡ Fisher & Porter, Hatborough, Pennsylvania—10 and 1017A 08F 1/16 12-4, Stainless Steel Ball.

any flowrator reading was thus obtained. This method of calibrating did not take into consideration the effect of gas expansion, which took place as the cold air entered the warm monel tube and hot investment; but, at a constant burn-out temperature, this should give comparable results for different investments. The heat of the molten metal was also a factor in the volume of gas present, but again comparative results were obtained. The specimen was made by customary dental procedures, using an investment containing hydrocal 28 per cent, cristobalite 15 per cent, and silica 57 per cent. The investment was mixed and poured into a ring on the adapter. Tests were made with or without a ring and with or without asbestos lining. When making tests

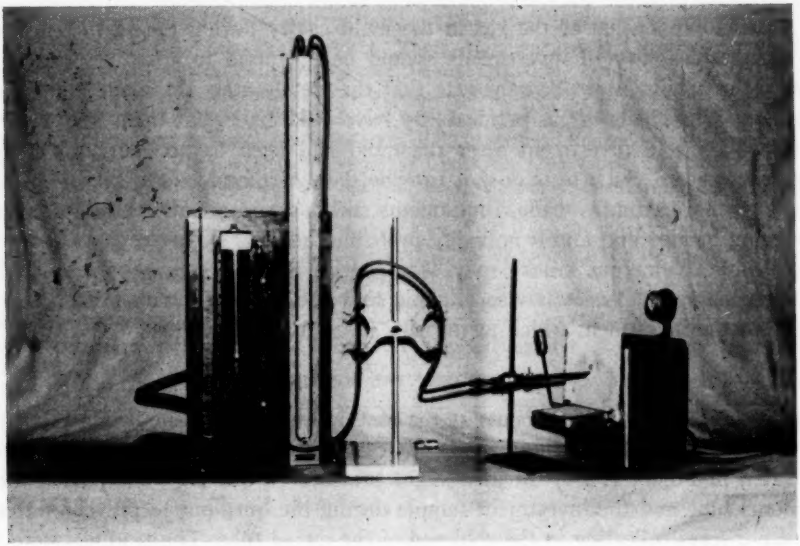

FIG. 1.—Complete permeability equipment with adapter and specimen in furnace

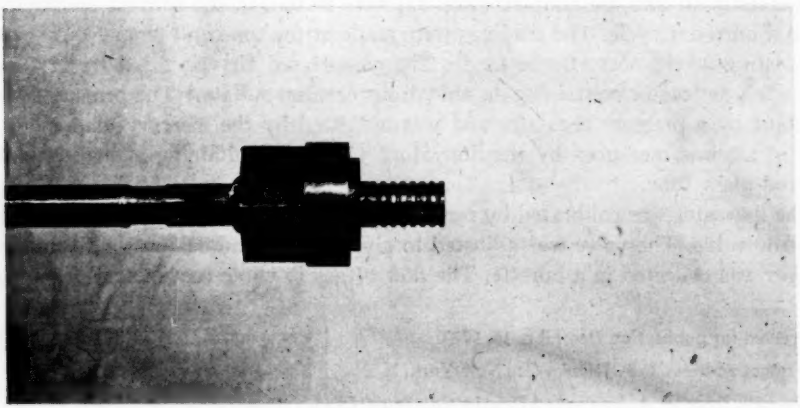

FIG. 2.—Monel adapter

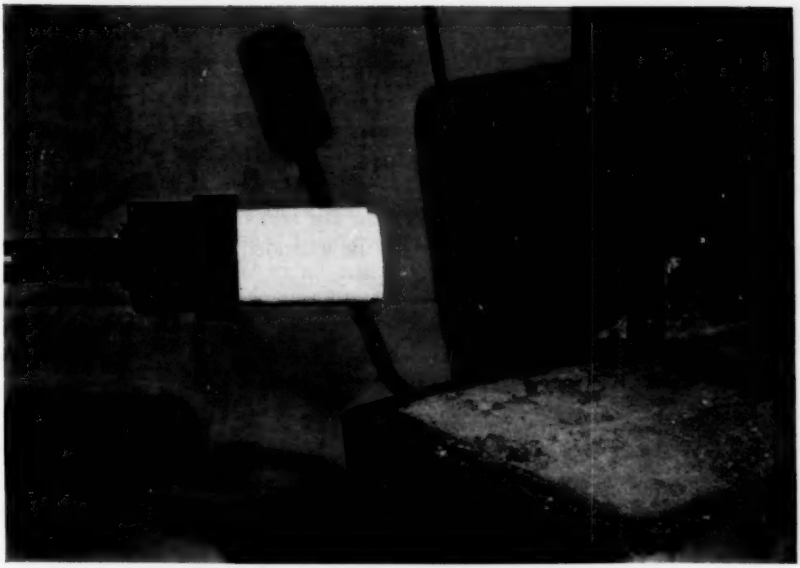

FIG. 3.—Adapter and investment sample before placing in furnace

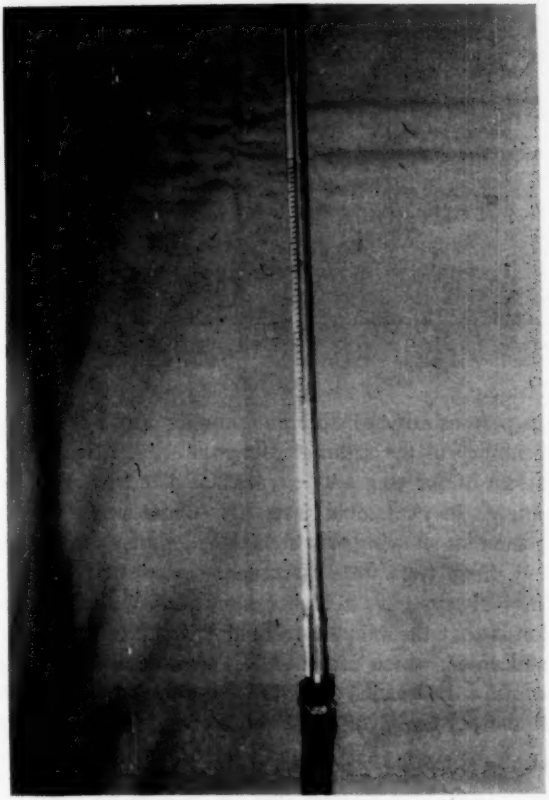

FIG. 4.—Flowrator with steel-ball float

on the investment with no ring, a temporary rubber ring* was employed which was removed after the investment had set and before the investment was placed in the furnace (Fig. 3). The investment was immersed in water for 1 minute before being placed in the furnace. A metal rod was placed in the adapter orifice before investing, to prevent the investment from entering the adapter. This equipment is designed so that a distance of $\frac{1}{2}$ inch is available between the end of the adapter tube and the extreme end of the ring. The metal rod may be extended out of the adapter, so that any thickness of investment less than $\frac{1}{2}$ inch is available. It is also possible to provide wax of any given thickness up to $\frac{3}{8}$ inch on the end of the rod to determine the effect of burned-out wax on permeability.

TABLE 1

GAS PERMEABILITY OF ONE INVESTMENT

	Thickness of Investment					
	¼ Inch		½ Inch		¾ Inch	
	Average	Range	Average	Range	Average	Range
Water added	123	100–130	152	135–65	.....	.....
No water added	105	88–120	.....	.....	.....	.....
Vacuum mix, water added	195	180–215	.....	.....	.....	.....
¼ inch wax, ¼ inch investment, water added	.....	.....	230	210–50	.....	.....
⅛ inch wax, ⅜ inch investment, water added	.....	.....	.....	.....	190	182–210
Water added	1 cc. per each ring, 50 gm. of investment					
Water/P ratio	26/100					
Mechanical mix	30 seconds, 600 rpm					
Formula	Hydrocal, 28 per cent; cristobalite, 15 per cent; silica, 57 per cent					
No metal ring employed	.....					

RESULTS

The variation in permeability of different samples was rather large. For instance, the range in permeability of ten samples using the same investment with ½-inch distance from the bottom of the ring and as constant a technique as possible amounted to 100–130 cc/minute. In occasional tests, the upper range was increased to 150 cc/minute, but, in samples showing this high range, numerous voids in the investment were always present. Such tests were discarded.

Vacuum investing† always gave a higher permeability than a non-vacuum mix. A possible explanation is that the vacuum technique broke up the large air bubbles into a multitude of small voids, which provided less restriction to the passage of air.

In some commercial investments employed for the water-added technique, it was also observed that the permeability decreased moderately with longer burn-out time

* Ransom & Randolph's aquascopic rubber ring.

† Barr vacuum investing unit, Torit Manufacturing Co., St. Paul, Minnesota.

and decreased appreciably with an increase in the burn-out temperature from 850° to 950° F. This may be explained by the fusion of some of the chemicals in the investment, sealing some of the passages.

The presence of wax in the investment increased the permeability even at 850° F. For example, $\frac{1}{4}$ inch of investment without wax had an average permeability of 152 cc/minute, and with the same thickness of investment containing $\frac{1}{4}$ inch of wax, the permeability was 230 cc/minute (Table 1).

With a permeability of 200 cc/minute (the volume occupied by a crown or inlay with an average value of $\frac{1}{4}$ cc.) the gas should be eliminated in 0.075 second, which is probably less than the time required to fill the mold. The molten metal heats the air and expands it so that the value may be over twice the volume of the wax, but the heated gas may also exhibit a greater flow rate through the investment.

It is also interesting to find that the permeability of investment before burn-out was negligible. No reading on the flowrator was ever obtained with "green" investment, indicating a minimal amount of permeability. When the wet investment was placed in the furnace at 850° F, the first permeability readings were obtained after about 10–15 minutes. This increased to a maximum at about 25–30 minutes and remained rather constant up to $1\frac{1}{2}$ hours. It would be interesting to correlate the short burn-out time with the gas porosity in castings.

SUMMARY

Equipment and technique are described for measuring the gas permeability of investment at burn-out temperatures and at pressures corresponding to those employed in dental practices.

It is difficult to obtain reproducible readings on different specimens. This may be due to the large variations in the amount of voids present in poured investments. Vacuum investing increased permeability. Burnt-out wax increased permeability. Longer burn-out time and higher temperatures were found to decrease the permeability of some investments. Using a water-added investment, the permeability through $\frac{1}{4}$ inch of investment should allow $\frac{1}{4}$ cc. of gas to escape in less than 1 second.

The Pattern of the Mineralization of Enamel

JAMES K. AVERY, ROGER L. VISSER, and DONALD E. KNAPP
The University of Michigan School of Dentistry, Ann Arbor, Michigan

Voluminous literature on the subject of enamel mineralization has been reviewed and published recently.[1, 2] Therefore, the author will not attempt to cite again the numerous individual contributions of the many investigators in this area. An evaluation of the past studies leads the reader to the conclusion that at least two general concepts of enamel mineralization have been developed and appear in the literature today. One concept postulates that mineralization of the enamel follows the pattern of formation of the enamel matrix from the dentino-enamel junction peripherally.[1–12] A second concept of enamel mineralization suggests that enamel matrix formation and final mineralization are separate processes, the latter not occurring until after the former is complete.[13–22] This latter concept indicates that calcification of enamel matrix begins at the cusp tips and proceeds cervically in a direction perpendicular to that of incremental deposition of the matrix.

Textbooks[23, 24] have adhered to the principles of the latter concept, stating that a primary influx of mineral salts occurs during matrix formation and that a secondary calcification process, in which three-quarters of the ultimate mineral content appears, occurs after matrix formation is complete. This secondary mineralization process, termed "maturation," is said to proceed non-incrementally from the tips of the cusps to the cervical region and at right angles to the long axis of the tooth.

Recently extensive investigations utilizing fluorescence and polarizing microscopy,[1] electron microscopy,[25, 26] isotopes,[27, 28] and microradiography,[2, 8–12] have revealed new and convincing evidence confirming the earlier beliefs[3–5] that enamel is mineralized from the dentino-enamel junction peripherally in the same or a similar pattern as that of matrix production. A recent study of the birefringence of developing enamel[29] has confirmed the microradiographic findings of a gradient of mineral concentration from the dentino-enamel junction peripherally. It is difficult, however, to evaluate the recent information in respect to whether or not total pre-eruptive mineralization has been accounted for. Whereas the autoradiographic and microradiographic techniques have shown clearly the relative distribution of mineral, they have not quantitatively assessed mineral content. These latest investigations have not, therefore, ruled out a secondary mineralization or maturation of enamel which has been postulated from quantitative chemical analysis of developing enamel.[18]

The present studies were carried out in an effort to determine the amount and dis-

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Parts of this investigation were reported before the I.A.D.R. and published in abstract form in the *J. D. Res.*, **38**:678, 1959, and **39**:712, 1960.

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tribution of mineral in all stages of enamel mineralization by using a combination of four techniques: microhardness tests, microradiography, microdissection, and microsubstitution.[30] It was believed that, by correlation of the findings from these methods, information would be gained concerning the processes involved in the mechanism of enamel mineralization and indicating whether or not there are two distinct stages in the calcification of enamel matrix.

MATERIALS AND METHODS

A group of 22 teeth was obtained at autopsy from four humans aged two and one-half, three, four, and four and one-half years. Another series consisting of 34 developing teeth was taken from a group of six Rhesus monkeys, one to three years of age. The teeth were selected for study according to the extent of formation and calcification as observed by conventional radiographs (Fig. 1). After dissection from the jaws, the

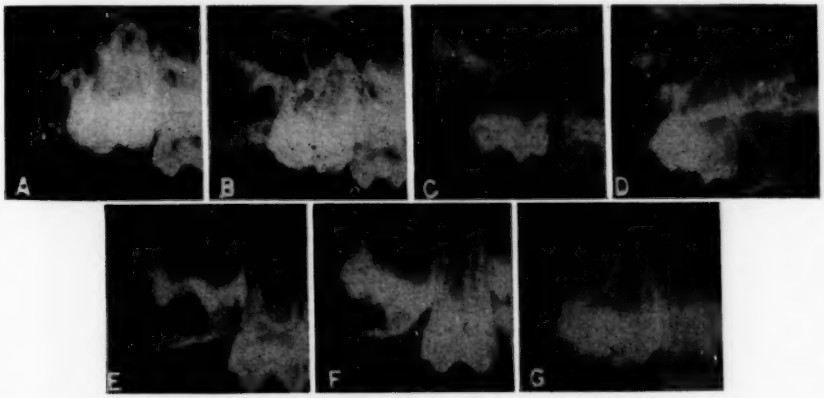


FIG. 1.—Radiographs of a representative series of monkey maxillary second permanent molars used in the study. They are arranged in developmental sequence from early stages of calcification of the cusps in *A* to a stage of eruption through the alveolar bone in *G*.

developing crowns were photographed (Fig. 2) and then dehydrated, embedded in quick-setting plastic (to prevent infiltration), and bisected by means of a water-cooled cutting disk.* One half of each tooth was cut into serial sections of approximately 75 μ for microradiographic study. The thickness of each section was measured with a micrometer to determine the proper time of radiographic exposure for each sample. The thin sectoins were stored in distilled water until ready to be radiographed. They then were dried and placed in direct contact with spectroscopic plates.† Microradiographs were taken at a voltage of 10 kv. This voltage gives X-rays of a wave length of approximately 2.2 A, which is the proper wave length for maximal absorption by the calcium ions. The sections were exposed to 30 milliamperes for 35–70 minutes, depending on the thickness of the section. The microradiographs were then developed in Kodak D-19 for five minutes at 68° F. Negatives of the plates were prepared, and enlarged prints made for study.

* Gillings-Hamco Thin Sectioning Machine, Hamco Machines, Inc., Rochester, New York.

† Kodak Type 649-0.

The other halves of the plastic-imbedded crowns were used for microhardness tests. These halves were cut into sections ½ mm. thick, affixed to plastic slides, and polished. The enamel then was tested for microhardness,* using a 25-gm. weight. Only uniform indentations showing a diamond-shaped impression with the long axis seven times greater than the width were used. In each sample a sufficient number of hardness measurements were made so that all areas of the enamel were evaluated. This resulted in as few as 35 measurements on young teeth with limited areas of sufficient hardness for measurement to as many as 100–125 measurements on more mature teeth. To facilitate interpretation of the pattern of hardness of each crown, the tooth sections were projected so that a magnified tracing of each section was obtained, and zones exhibiting a range of 30 Knoop Hardness Numbers (KHN) then were outlined on these diagrams.

In another series, developing human and monkey teeth were studied by microdis-

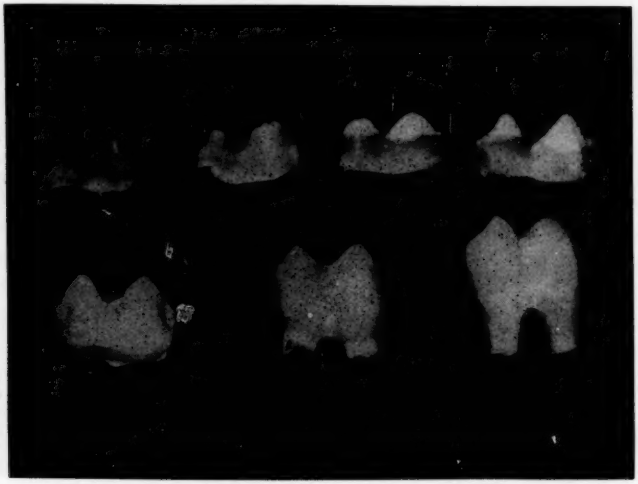
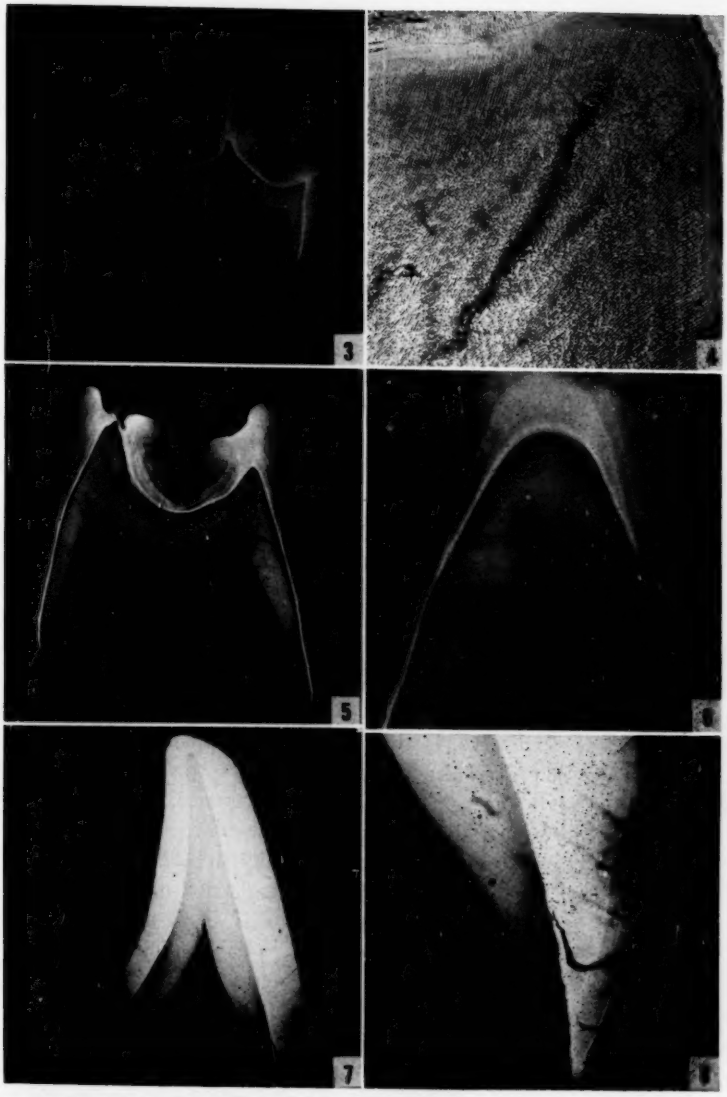


FIG. 2.—Photograph of the series of developing second molar crowns shown in Fig. 1, now dissected from the jaws. Observe the "formalin brown" (ref. 5) in contrast to the white enamel, which has led some authors to suggest the two-stage enamel development and calcification theories.

section and microsubstitution.[30] These teeth were sectioned at 100–200 μ, placed in Soxhlet extractors, and extracted with 80 per cent ethylenediamine at 118° C. for 72 hours. After extraction of organic matter they were washed with distilled water. One group of sections was immersed in water, and the enamel rods were dissected with finely drawn glass probes and studied under a stereomicroscope. The water was removed from the remaining organic-free sections in a 60° C. oven. Then these sections were infiltrated under vacuum with an unsaturated polyester resin which filled the spaces formerly occupied by the organic matter and water. After the plastic had cured, the block was polished in order to expose the tooth section and was placed in 0.1 N hydrochloric acid to remove all mineral salts. After decalcification was complete, all that remained was a plastic skeleton which represented the location and relative concentration of organic material and water in the enamel and dentin and could be directly visualized and photographed.

* Tukon Microhardness Tester with a Knoop Diamond Indenter.



FIGS. 3–8.—**Fig. 3: Microradiograph of mesiodistal section of young monkey molar teeth illustrating the radiodense line along the dentino-enamel junction and small radiopaque projections at the peaks of each cusp. (Orig. mag. ×13.) Fig. 4: Transmitted-light photomicrograph of decalcified young enamel of a developing monkey molar, illustrating the rod pattern at the center of a cusp. The rows of cross-sectioned rods which appear dark in transmitted light correspond with the radiopaque zone seen in the preceding figure. (Orig. mag. ×150.) Fig. 5: Microradiograph of a human molar at a more advanced stage of mineralization than that shown in Fig. 3. The incremental lines appear more radiopaque than the adjacent enamel matrix. The areas along the dentino-enamel junction and the tips of the cusps appear to be the most highly mineralized areas. Defects due to grinding the specimens thin can be seen at the tip of the left cusp. (Orig. mag. ×13.) Fig. 6: Microradiograph of a human molar illustrating an area of increased mineral density peripheral to the dentino-enamel junction. This increase in mineral content appears to mask the incremental lines. (Orig. mag. ×60.) Fig. 7: Microradiograph of a human incisor, illustrating a more advanced state of mineralization. The enamel in this specimen appears uniformly radiodense except at the cervical areas of the crown. (Orig. mag. ×13.) Fig. 8: A higher magnification of the cervical area of the crown shown in Fig. 7, illustrating the advancing mineralization in an incremental or nearly incremental pattern. The increased density of the mineral has masked all but a few of the radiopaque incremental lines. (Orig. mag. ×40.)**

A final series of eight human and eight monkey teeth was collected for the purpose of comparing pre-eruptive and posteruptive hardness. The pre-erupted human teeth were bony-impacted third molars with radiographic evidence of fully formed roots. It was presumed from this latter characteristic that the enamel of these teeth was calcified to its normal pre-eruptive extent. The series of human erupted teeth consisted of maxillary and mandibular first molars. The monkey pre-erupted teeth were maxillary and mandibular second molars, and the erupted teeth were maxillary and mandibular first molars. Each of the human and monkey molars was from a different subject. All the teeth were imbedded in plastic and then sectioned. Microhardness determinations were made in the same manner as for the first group of teeth.

RESULTS

Microradiography.—In the youngest human and monkey teeth studied by microradiography, only a narrow radiopaque line measuring 35–50 μ in width could be seen along the dentino-enamel junction of the entire crown (Fig. 3). This narrow band, which is the first indication of enamel mineralization, appeared even in the more cervical regions of the crown, where the amount of matrix and the length of time that it had been deposited were much less than at the cusp tips. The next area of increase in mineral content was at the cusp tip, spreading laterally and peripherally from the narrow spike which extended at right angles from the dentino-enamel junction toward the tips of the enamel cusps. These radiodense spikes were found to be prominent in developing monkey enamel and to correlate with the enamel-rod orientation of the sharply pointed cusps of monkey teeth. In the exact center of each developing cusp two adjacent Schreger bands or groups of enamel rods curved toward each other, resulting in a row of rods sectioned in the transverse axis. A difference in X-ray absorption was seen along this row, as well as differences in optical light paths seen by transmitted light (Fig. 4). The effect was due, at least partially, to the morphology of the rod structure but also to an increase in mineral density, since X-ray absorption increased further in this area in slightly older cusps. In human teeth most cusps were not as pointed as those of monkey molars, so that the area of mineralization extending from the dentino-enamel junction appeared more gently curved (Fig. 5). Gradually, the incremental lines (lines of Retzius) appeared more highly mineralized than the adjacent matrix (Fig. 6). Further mineralization from the dentino-enamel junction peripherally then masked the incremental lines in the area of the cusp tips (Fig. 7). The same process of calcification occurred later in the more cervical portions of the developing crown (Fig. 8). During the early stages of calcification a high-magnification microradiograph revealed radiodense enamel rods and radiolucent interrod* substance. Measurement of this uncalcified area between the rods showed that it was wider in young, calcifying enamel than it was in more completely calcified enamel (Figs. 9 and 10).

Thus it would appear that mineralization of young developing enamel followed an incremental or nearly incremental pattern from the dentino-enamel junction peripherally, similar to the pattern of matrix deposition. The mineral did not appear to be limited to a certain number of increments behind matrix formation but appeared to

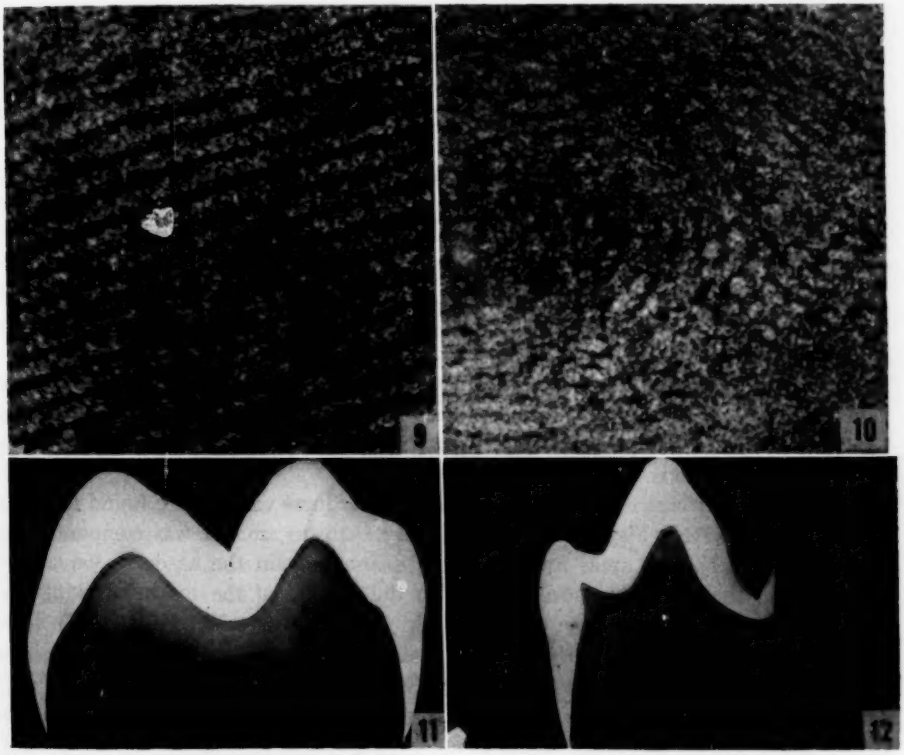
* The term "interrod" substance is preferred to "interprismatic" substance because the prismatic (eight-sided) shape of enamel rods has been disproved (see ref. 25).

diffuse from the areas of oldest matrix into the younger matrix. Mesial cusps of all molar teeth studied appeared to be more advanced in development (i.e., more highly mineralized) than the distal cusps (Fig. 3).

Microradiographs of young, calcifying enamel matrix presented varied characteristics. For example, in some specimens alternate Schreger bands had different degrees of radiodensity. Other specimens showed areas of unexplained opacity or lucidity, perhaps due to pathology of the enamel. For microradiographic studies, the sections should be ground exactly plano-parallel. Since this is difficult, if not impossible, this factor could have accounted for most of the variations from the general pattern.

After the radiopaque incremental lines had become obscured, the enamel appeared uniformly radiopaque (Figs. 11 and 12). No further information on differences in mineral density of these teeth could be gained by microradiography, so the other halves of each of these teeth then were tested for microhardness.

Microhardness.—Of the entire series of human and monkey teeth that were micro-



FIGS. 9–12.—Fig. 9: Microradiograph of young calcifying enamel matrix, illustrating the calcified rod substance in contrast to the non-calcified interrod substance. The microradiograph was magnified by means of photographic enlargement. (Orig. mag. ×200.) Fig. 10: Microradiograph of older calcifying enamel matrix. Less radiolucent interrod substance is visible. The minute black dots in the photograph probably represent spaces between the more radiopaque groups of crystals. Test analysis of the spectroscopic plates revealed no grain size of this magnitude. (Orig. mag. ×600.) Fig. 11: Microradiograph of the developing crown of a human molar which reveals a uniform distribution of mineral throughout the enamel. (Orig. mag. ×13.) Fig. 12: Microradiograph of the enamel of a monkey molar, revealing uniform radiodensity throughout. (Orig. mag. ×13.)

radiographed, only the oldest, uniformly radiopaque teeth were of sufficient hardness to gain accurate indentations with the Tukon Hardness Tester. Microradiographs obtained at 10 kv and an exposure of 30 milliamperes for 35–70 minutes showed enamel to be uniformly radiopaque at a Knoop Hardness Number (KHN) of 100 or higher. It would appear that the microradiographs of mineralizing teeth used in this study revealed a distribution pattern of only a small amount of the total mineral if we consider that adult human enamel hardness is 343 ± 23 KHN.[31] These observations are shown in microradiographs (Figs. 11 and 12) and in Figures 14 and 18, which represent hardness patterns of the same teeth.

The first areas of measurable hardness in both human and monkey enamel were along the dentino-enamel junction and in a narrow zone adjacent to this at the tips of the cusps. The 35–50 μ band of enamel along the dentino-enamel junction had hardness values ranging from 150 to 280 KHN. This band was found to be consistently harder in the cusp than at the more cervical aspects of the crown. Fifty microns away from the dentino-enamel junction the enamel was too soft to be measured except in the region of the cusp tips (Fig. 13). No patterns indicating relative mineralization could be obtained on this tooth. Measurements at the cusp tip in a slightly more calcified tooth revealed hardness values decreasing from 280 to 30 KHN as the indenter was moved toward the periphery of the cusp from the band along the dentino-enamel junction (Fig. 14).

The next tooth in the series was more advanced developmentally and exhibited a greater range of microhardness values. Although the band of mineralized enamel at the dentino-enamel junction was similar in hardness to that of the previous tooth, a great deal more mineral was apparent peripheral to this band. In this sample the hardness values ranged from 270 KHN to very soft areas of 0–30 KHN at the cervical area of the crown (Fig. 15). The map of the nine zones of decreasing hardness values presents an incremental appearance of mineral distribution in the developing crown very similar to the incremental pattern of matrix formation. It should be remembered that these zones pertain only to 30-KHN ranges of microhardness and are not based on demonstrable morphologic divisions of enamel matrix such as those separated by the incremental lines of Retzius.

A study of other crowns followed, in which the hardness values were found to range from 0 to 400 KHN (Figs. 16 and 17). In these crowns, zone 1 was comparable in hardness to the hardest areas found in adult enamel. Again the hardness values decreased from the dentino-enamel junction to the surface of the tooth following the chronologic incremental pattern of matrix formation. These mineralizing teeth illustrated the maximum range in hardness of enamel found in this study and thus show the greatest number (10) of zones of hardness.

In the next older specimen the range of microhardness values decreased as the size of the areas within the range of adult hardness increased (Fig. 18). The hardest areas, located at the cusp tips, were found to be uniformly mineralized. The less hard areas, located in the grooves between the cusps and at the cervical region of the crown, still exhibited an incremental pattern of hardness, although no areas of less than 90 KHN were found.

Following this, a specimen was selected which was in the process of eruption but had not emerged into the oral cavity. The measurements indicated that all areas of the

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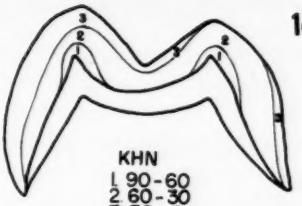

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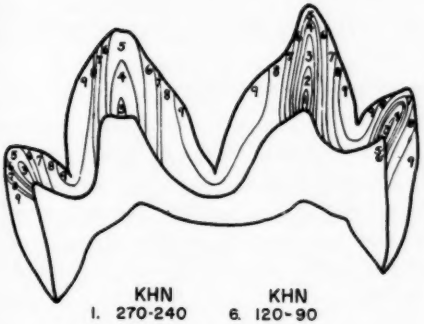

KHN		KHN	
1.	270-240	6.	120-90
2.	240-210	7.	90-60
3.	210-180	8.	60-30
4.	180-150	9.	30-0
5.	150-120		

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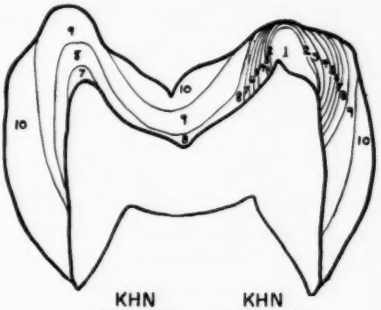

KHN		KHN	
1.	400-270	6.	150-120
2.	270-240	7.	120-90
3.	240-210	8.	90-60
4.	210-180	9.	60-30
5.	180-150	10.	30-0

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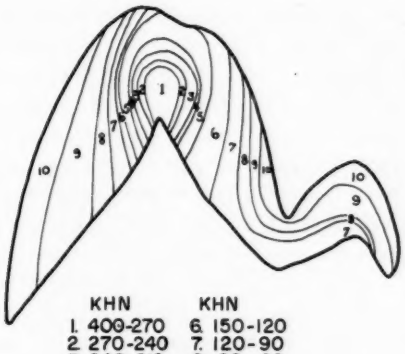


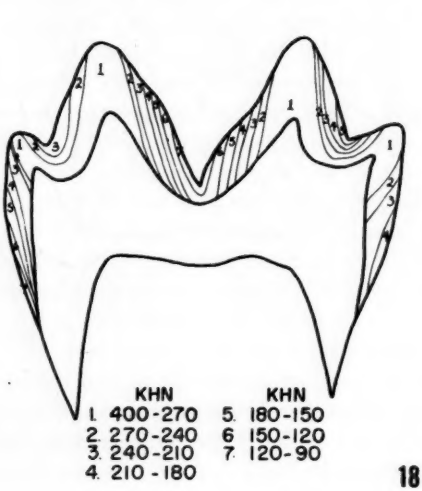
KHN		KHN	
1.	400-270	6.	150-120
2.	270-240	7.	120-90
3.	240-210	8.	90-60
4.	210-180	9.	60-30
5.	180-150	10.	30-0

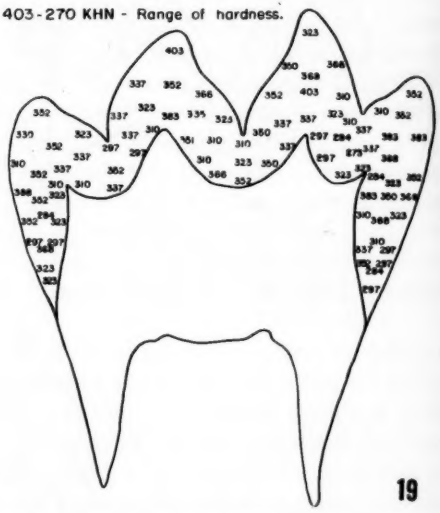
17

FIGS. 13–17.—**Fig. 13: Projection diagram of a mesiodistal section of young monkey molar, illustrating that the only enamel matrix of sufficient hardness to gain accurate indentation was at the tips of the cusps. The enamel in this area measured 0–30 KHN. (Orig. mag. ×13.) Fig. 14: Projection diagram of a buccolingual section of a young human mandibular second molar, illustrating three areas of different hardness. Zone 1 near the dentino-enamel junction of the cusp tips was the hardest zone. This diagram and the microradiograph shown in Fig. 11 are of sections taken from the same tooth. (Orig. mag. ×13.) Fig. 15: Projection diagram of a mesiodistal section of a monkey molar, indicating a more highly calcified tooth than that shown in Fig. 14. The hardness values, grouped in increments of 30 KHN, indicate the pattern of matrix formation. Zone 1 is in the lower limits of the range of adult hardness. (Orig. mag. ×13.) Fig. 16: Projection diagram of a buccolingual section of a human maxillary permanent first molar, illustrating a slightly more highly calcified tooth than shown in Fig. 15. Zones 1 and 2 are in the range of adult hardness. (Orig. mag. ×13.) Fig. 17: Projection diagram of a buccolingual section of a human permanent premolar, illustrating a tooth of hardness similar to that in Fig 16. (Orig. mag. ×13.)**

enamel were within the range of adult hardness and that there was no incremental pattern of mineral density (Fig. 19).

It was then of interest to determine the extent of possible posteruptive mineralization. Eight human teeth—four pre-erupted and four erupted—were measured (Table 1). The mean hardness of enamel of the pre-erupted teeth was found to be 422 ± 53 KHN and that of enamel of the erupted teeth was 403 ± 53 KHN. In a similar series of monkey teeth the mean hardness figures were 401.5 for pre-erupted enamel and 406.5 for erupted enamel. It would appear that very little mineral, if any, contributed





FIGS. 18–19.—Fig. 18: Projection diagram of a mesiodistal section of a monkey molar slightly more calcified than the tooth depicted in Fig. 17. The range of hardness values has decreased, with the least hard area measuring 90–120 KHN. Observe the incremental nature of the hardness pattern. This section was taken from the same tooth as that shown in Fig. 12. (Orig. mag. ×13.) Fig. 19: Projection diagram of a mesiodistal section of a monkey molar in the intraosseous stage of eruption. All measurements of enamel are within the range of adult hardness, and there is no incremental pattern of hardness. (Orig. mag. ×13.)

TABLE 1

HARDNESS VALUES OF HUMAN PERMANENT TEETH*

Tooth	Pre-erupted				Tooth	Erupted			
	Periphery		All Other Areas			Periphery		All Other Areas	
	KHN ±S.D.†	No. of Indentations	KHN ±S.D.	No. of Indentations		KHN ±S.D.	No. of Indentations	KHN ±S.D.	No. of Indentations
3d max. molar....	428±54	32	386±57	80	1st max. molar...	411±50	21	317±64	88
3d mand. molar..	503±51	34	421±37	79	1st max. molar...	433±57	21	398±50	59
3d mand. molar..	453±62	26	409±57	87	1st mand. molar..	453±60	27	397±36	87
3d mand. molar..	387±64	20	391±61	67	1st mand. molar..	417±51	24	397±53	60
Mean.....	443±58	28	402±53	78	Mean.....	429±55	23	377±51	74

* All teeth from different individuals; all readings considered.
† S.D. = Standard deviation.

to an increased hardness posteruptively. It was also observed in all cases that the surfaces of both human and monkey pre-erupted and erupted teeth proved to be harder than the underlying enamel.

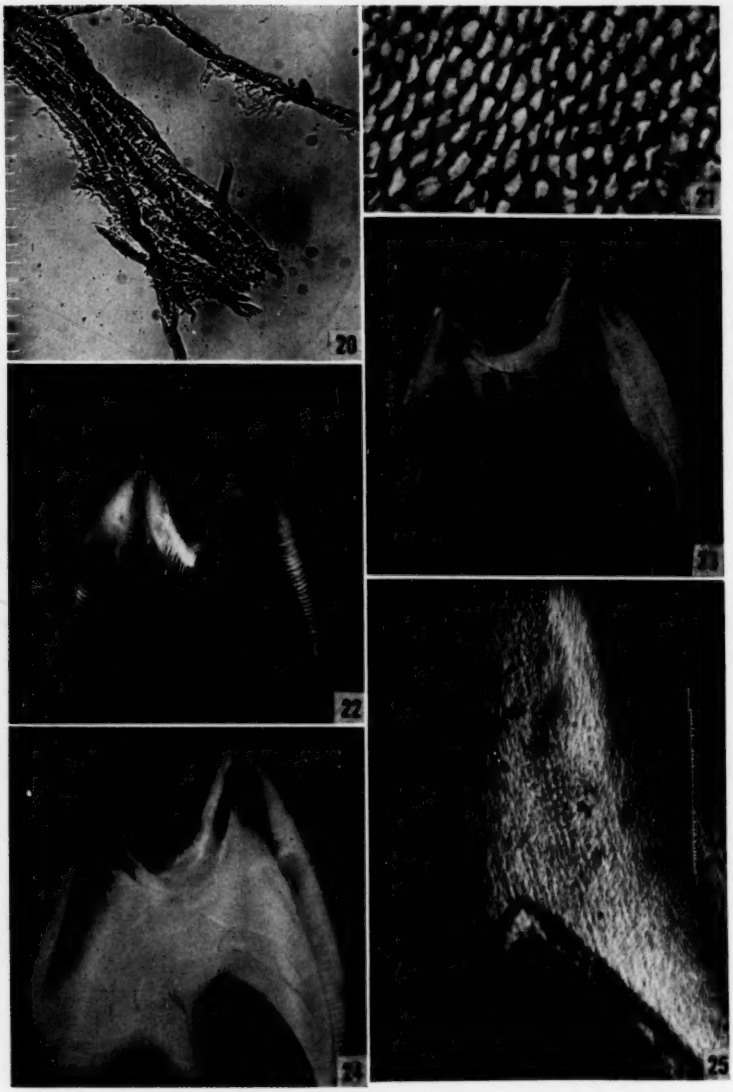
Microdissection and microsubstitution.—The ethylenediamine-treated specimens of human and monkey enamel were immersed in water and dissected under a stereomicroscope with finely drawn glass needles. It was found that the organic-free matrix of developing enamel which had not yet reached its adult thickness offered relatively little resistance to microdissection and that the individual rods or groups of calcified rods could be separated from each other with ease (Fig. 20). In the areas where the full thickness of the enamel had been attained, the rods could not be separated one from another. Decalcified developing enamel exhibited an opposite picture in which the rods were missing and the interrod substance was retained (Fig. 21).

The ethylenediamine-treated rods then were measured, and it was found that at the dentino-enamel junction the average diameter of enamel rods was 4.9 μ, whereas the diameter at the other end of the rod, near the surface of the enamel, measured 5.1 μ. It is not known whether the entire rod substance was present, however. This difference between the rod diameters at the dentino-enamel junction and at the periphery was noted to be consistent in various regions of the crown. However, the differences noted in rod diameter are within the range of experimental error.

A light-microscope study then was made of the series of developing crowns of human and monkey teeth in which the organic matter and water had been removed and replaced by polymers of small molecular size. After decalcification these specimens were examined by both reflected and transmitted light. Areas of high mineral content were found to contain very little plastic, whereas the less mineralized areas contained proportionately more plastic. The first area in enamel to exhibit noticeable mineralization was a narrow band along the dentino-enamel junction of the youngest tooth studied (Fig. 22). The next older specimen revealed a triangular area of more calcified enamel in the cusp tips, extending peripherally from the dentino-enamel junction for half the thickness of the enamel (Fig. 23). In an older specimen mineralization appears to have proceeded peripherally from the dentino-enamel junction not only at the cusp tips but along the sides of the cusps as well (Fig. 24). Mineralization, therefore, was advanced in all the areas of the earliest-deposited matrix. It can be observed that only small amounts of plastic, representing the organic interrod substance, were still present in the mineralized areas (Fig. 25). It is apparent that the location and degree of mineralization as ascertained by this technique correspond with those demonstrated by the microradiographic and microhardness determinations.

DISCUSSION

The appearance in the microradiographs of a strongly radiopaque line in enamel along the dentino-enamel junction confirms the findings of other investigators.[2, 6, 8–12, 19, 20] The origin of this highly mineralized 35–50 μ-wide band may be from the tissue fluid of the pulp or the enamel organ or from both, and it is possible that it plays some role in succeeding enamel mineralization. This zone rapidly attains adult hardness, however, and therefore would inhibit the movement of minerals from the pulp to contribute to further enamel mineralization. An interesting factor is that two structurally different fiber types, each with crystals of a different size, are inter-



FIGS. 20–25.—Fig. 20: Ethylenediamine-extracted enamel of a developing human tooth. During early calcification of the enamel matrix the rods could be separated easily, illustrating the large proportion of organic material in the interrod substance. Scale at left indicates that the rods are approximately 5μ in diameter. (Orig. mag. $\times$380.) Fig. 21: Transmitted-light photomicrograph of decalcified young enamel of a developing human molar, illustrating the remaining interrod substance and the loss of calcified rod structure. Fig. 22: A reflected-light photomicrograph of a molar tooth after removal of organic matter and water and replacement by plastic of small molecular size. After decalcification, zones of high mineral (low plastic) concentration were revealed along the dentino-enamel junction. (Orig. mag. $\times$13.) Fig. 23: A reflected-light photomicrograph of a slightly more highly mineralized tooth prepared in the same manner as that shown in Fig. 22. High mineral and low plastic concentration is seen clearly as the dark area extending peripherally from the dentino-enamel junction of the cusp tips. (Orig. mag. $\times$13.) Fig. 24: A somewhat more calcified tooth prepared in the same manner as those in Figs. 22 and 23. Observe the lack of plastic, indicating high mineral content at the cusp tips and in the areas adjacent to the dentino-enamel junction along the sides of the cusps. (Orig. mag. $\times$13.) Fig. 25: A high-magnification, transmitted-light photomicrograph of the right cusp tip shown in Fig. 23. The dark and light areas are reversed from the previous pictures, so that the dark areas in this transmitted-light picture are the plastic, representing the organic material and water. The light areas indicate high mineral, low plastic concentration where calcification is advanced. (Orig. mag. $\times$125.)

mingled in the inner portion of this zone.[25] The approximately 50-A-wide fibrils of the enamel matrix[26] have apatite crystals of 400–500 A in width,[25] whereas the 600-A-wide collagen fibrils of dentin[25] have several smaller crystals within the diameter of a single fiber.[26] Nowhere else in the human body is the specific nature of crystal formation on a specific fiber more clearly demonstrated than at the dentino-enamel junction. The present evidence that mineralization follows enamel matrix formation in a nearly incremental fashion suggests that time may be an important factor in the changes which the matrix fibers undergo before crystal nucleation and growth can occur. Changes in the matrix, such as loss of water and organic material[32] or dehydration and condensation,[33] are part of the mineralization process. However, other less easily determined mechanisms, such as phosphorylation, molecular transformation of sulfated polysaccharides, or electrostatic changes of the organic matrix, undoubtedly occur before mineralization can proceed. [34, 35]

It has been noted that the interrod matrix is formed before the rod matrix[25, 36] and contains some mineral.[37] However, the rod matrix becomes completely calcified before the interrod matrix calcifies. The finding of organic interrod substance after decalcification of mineralizing enamel has been noted by most dental histologists and was noted in the present study. Microradiographs showed that radiolucent areas between the rods during early mineralization were wider than such areas in more completely calcified enamel. It is possible that if the rod calcifies from the center peripherally and if it is not completely calcified at this stage of development, the area between the calcified portion of the rods is in part uncalcified rod substance, as well as interrod substance. Figures 9 and 10 would appear to support the theory that the rods calcify from the center peripherally. Microdissection of organic-free developing enamel indicated that the interrod substance must have contained a relatively large proportion of organic matter which was dissolved by the ethylenediamine, thus allowing separation of the rods. The rods themselves, however, were sufficiently calcified that, in spite of the loss of their organic matrix, their general morphology was retained. This observation is significant in view of the following. First, in the development of enamel, the interrod space is the logical route for calcium and phosphate ions to diffuse along and around the entire length of the developing enamel rod, thus allowing the rod matrix to calcify from the dentino-enamel junction peripherally. Then, after the enamel rods are completely calcified, the interrod matrix becomes completely calcified. Second, in caries of enamel the interrod substance is the first area to be altered.[38–41] It is of interest that the steps in enamel destruction are the same as those in enamel formation but in reverse order. It is therefore important now to concentrate investigative effort on determining the basic differences in the structure of the mineralized rod and interrod substances. Differences in solubility of these two may be due to such factors as the difference in degree of calcification of the rod and interrod substances, higher carbonate concentration of the interrod substance, and/or the difference in orientation of the interrod fibers and thus the crystal structure as compared with that of the enamel rod.[25, 36] The sum of these differences results in a mineral-fiber bond that is more susceptible to initial acid attack. It is difficult to explain why the sections in the present study exhibited increased radiopacity along the incremental lines or alternate Schreger bands. Such differences in radiodensity in these areas have been reported.[10, 20, 42] There is, perhaps, a different explanation for each of these observed variations. The

increased radiodensity of the incremental lines may represent a phase in the mineralization process indicating an area of increased crystal growth. This relative difference in mineral density between the incremental lines and adjacent enamel matrix gradually disappears as mineralization proceeds. This observation in the present study confirms the findings of Allan.[10] Therefore, the incremental lines of Retzius are developed initially as zones of higher mineral content at least as high as that of adjacent areas. Gustafson,[43] who carried out extensive studies on adult human enamel, noted that these lines are not zones of lower mineral content, as some authors[23, 24] have contended. The difference in X-ray absorption in alternate Schreger bands could possibly be due to the amount of uncalcified interrod substance penetrated by the X-rays.

The technique of microhardness has proved successful in illustrating that the complement of mineral in adult enamel is laid down in an incremental or nearly incremental manner following the pattern of matrix formation. There is, therefore, no possibility of a secondary maturation process at right angles to the incremental pattern which has been said to account for three-quarters of the mineral content of enamel.[24] Weinmann, Wessinger, and Reed,[18] in precise analyses of the calcium content of the crown tip and the middle and cervical areas of developing enamel, undoubtedly would have illustrated this if it had been technically possible for them to obtain samples of enamel from both the dentino-enamel junction and the periphery of enamel in each of those three areas. The artificial boundaries separating matrix formation and calcification therefore should be eliminated, as well as the term "maturation of enamel" which has been used to imply a secondary process of mineralization. We should, instead, orient our thinking to the intimate relationship of the fibrous matrix and mineral, in order to gain further information on the complex mechanisms involved in enamel crystal formation and growth.

Several observations concerning dentin formation were made during the course of this study. Although dentin matrix formation is known to begin prior to enamel formation,[23, 24] dentin matrix is deposited much more slowly than the enamel matrix in the young developing crown (Figs. 3–8). After enamel matrix deposition is complete, the thickness of the dentin equals and then exceeds that of the enamel (Fig. 18). This finding obviously does not support the daily appositional rhythm described by Schour,[23] since the two types of matrices must have different rates of incremental deposition. It was also noted during the microhardness studies that young, developing dentin near the dentino-enamel junction was only about half as hard as adult dentin. Therefore, further mineralization than that which occurs along the predentin-dentin calcification front must take place. This supports the recent autoradiographic findings of Kumamoto and Leblond[28] on interstitial calcification of the dentin.

The enamel of human and monkey erupted molars was not significantly harder than that of the pre-erupted molars. The mean hardness value obtained from 387 measurements in four erupted human first molars was found to be 403 ± 53, which is within the range of the findings of Craig and Peyton[31] of 343 ± 23 KHN. The mean hardness value of 422 ± 55 obtained from 425 measurements of the four pre-erupted human third molars would seem to indicate that they are slightly harder. This difference, however, is not significant in view of the size of the standard deviation. If there was a definite posteruptive increase or decrease in the mineral content of the tooth, it was believed to be too small to be identified in the present analysis. It is probable that

microhardness tests cannot give the final answer to the question of posteruptive mineralization unless an extremely large sample of teeth is studied. The range of hardness values is so great in comparing one crown with another and even within a single crown that the relatively small amount of mineral that may be deposited posteruptively cannot be identified as such. On the other hand, both the pre-erupted and the erupted teeth studied showed a definite increase in hardness at the enamel surface. This characteristic in adult erupted teeth has been noted by a number of investigators.[44–47] It has been observed also in microradiographs of developing enamel.[8, 10, 12, 29] In the present study, microhardness tests confirmed the findings of increased hardness at the enamel surface in both pre-erupted and erupted teeth. This would point to the conclusion that enamel surface hardness is due partially, if not entirely, to pre-eruptive developmental processes.

The technique[30] for substituting small-molecular-size polymer for the previously removed organic material and water in the series of developing crowns substantiated the evidence offered by the microradiographic and microhardness tests that teeth calcify from the dentino-enamel junction peripherally.

Although there are some slight differences in interpretation of the findings of recent investigations as to whether enamel mineralization occurs in one phase[8] or in several phases,[11, 12] there is agreement among these investigators that the pattern of mineralization of enamel follows closely that of matrix formation. Therefore, the area of initial matrix formation at the dentino-enamel junction of the cusp tip is the site of the first mineralization and the cervical area is the site of the last in every case. The findings of the present investigation further support these conclusions by accounting for the distribution of enamel mineral over the entire pre-eruptive period of enamel calcification.

SUMMARY

An investigation using microradiographic, microhardness, microdissection, and microsubstitution techniques was made to gain information about pre- and posteruptive calcification of enamel in 72 human and Rhesus monkey teeth. The following observations were made.

The general pattern of enamel matrix calcification followed and was closely related to the pattern of matrix formation, in disagreement with the theories of two-stage matrix formation–maturation processes.

A band along the dentino-enamel junction reached adult hardness during early matrix formation. The remainder of the matrix exhibited a gradient in radiopacity and hardness from the dentino-enamel junction peripherally which followed the same or a very similar pattern as the incremental pattern of enamel matrix formation. The enamel-rod matrix calcified before the interrod substance, the latter thus presenting pathways for diffusion of tissue fluid containing calcium-phosphate ions, during the mineralization process.

The appearance of radiopacity along the incremental lines was a characteristic of early mineralization. No evidence of a posteruptive increase in mineral content was demonstrated by the microhardness technique. Surface hardness was a characteristic of unerupted as well as erupted teeth.

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The Effect of the Administration of Tetracycline on the Development of Teeth

GERRIT BEVELANDER, GLORIA K. ROLLE, and SIDNEY Q. COHLAN

Department of Histology, New York University College of Dentistry, and the Department of Pediatrics, New York University Medical Center

Several investigators[1–9] have shown that the tetracyclines complex with several metallic ions. It has also been shown that tetracycline or a fluorophore of this drug is incorporated into growing bones.[4–5] In our own studies[6–7] it was shown that not only is the drug incorporated in developing skeletons, but that at appropriate levels a marked inhibition of skeletal development also occurs.

The present study was designed to amplify our previous findings, to ascertain the effect of the administration of tetracycline on developing teeth. We selected young rats 2 weeks old for this purpose, since at this age both incisors and molars undergo rapid growth and mineralization—a situation somewhat comparable to that which obtains in the human when the deciduous and mixed dentition are being formed.

MATERIALS AND METHODS

Two litters, each consisting of seven rats 2 weeks of age, were injected daily with 5 mg. of tetracycline hydrochloride* (achromycin) dissolved in 1 ml. of distilled water on 5 successive days. In addition, one rat of each litter was injected with distilled water only. The teeth of these rats served as controls.

One of the litters was sacrificed 1 day following the last injection; the other, 1 week after the last injection of the drug. The heads were removed and fixed in a mixture of 95 per cent ethyl alcohol and 5 per cent neutral formalin. Thirty-six upper incisors were removed, sectioned by grinding, and subsequently examined. Corresponding blocks of molars were similarly sectioned and examined. A total of 16 jaws, containing 32 incisors and 96 molars in all, was used in this study. The sectioned teeth were subsequently examined with the aid of a compound microscope in both visible and ultraviolet light. In visible light, one can usually identify tetracycline by its characteristic yellow-brown color. In the presence of ultraviolet light, tetracycline exhibits a characteristic yellow-gold fluorescence. A fluorescence lamp† with high-pressure mercury source was used with appropriate filters to assess the tetracycline-induced fluorescence in the teeth.

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† Leitz Fluorescence Lamp CS-150.

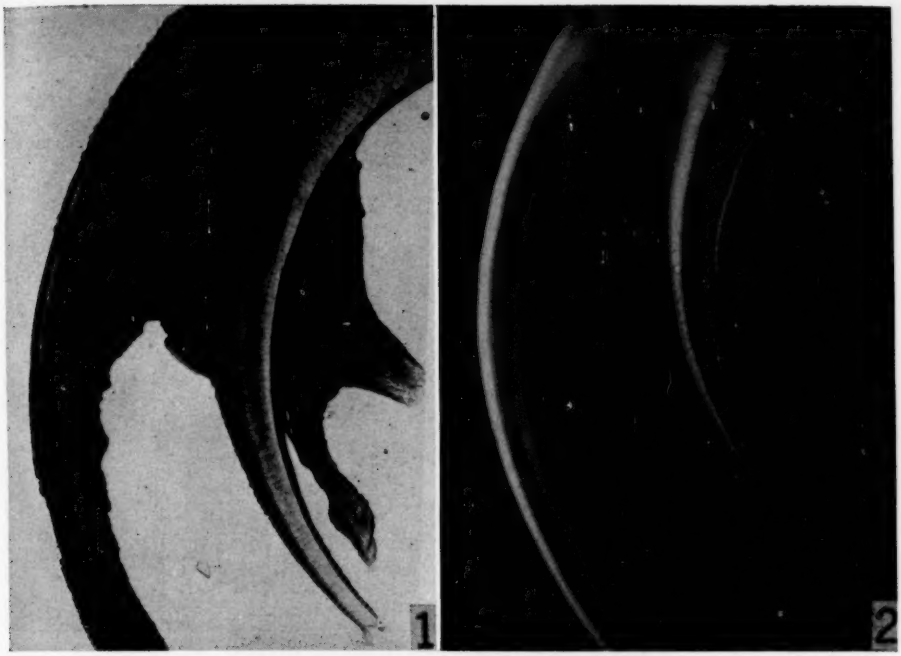

FIG. 1.—Longitudinal ground section of incisor of specimen injected on 5 successive days with tetracycline. (Low power, visible light.)

FIG. 2.—Same section as in Fig. 1 photographed in ultraviolet light. Note white (fluorescent) bands in dentin. Fluorescence also shown in alveolar bone.

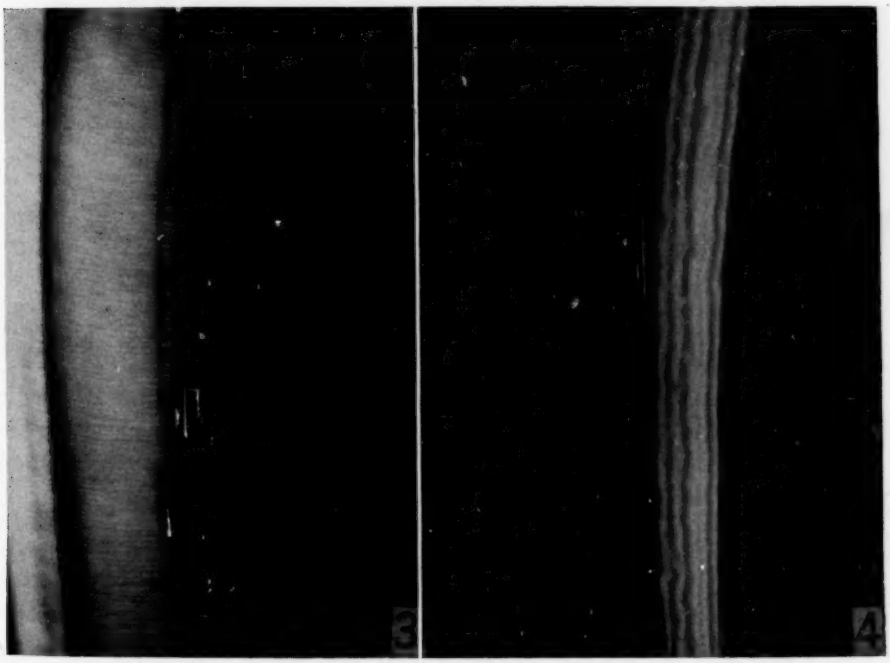

FIG. 3.—Part of dentin shown in Fig. 1 at higher magnification. Note 5 distinct bands in center of dentin strip, indicative of interference with calcification of the matrix. (Visible light.)

FIG. 4.—Corresponding section of dentin photographed in ultraviolet light. Note the 5 brilliant fluorescent bands in the dentin indicating presence of fluorophore.

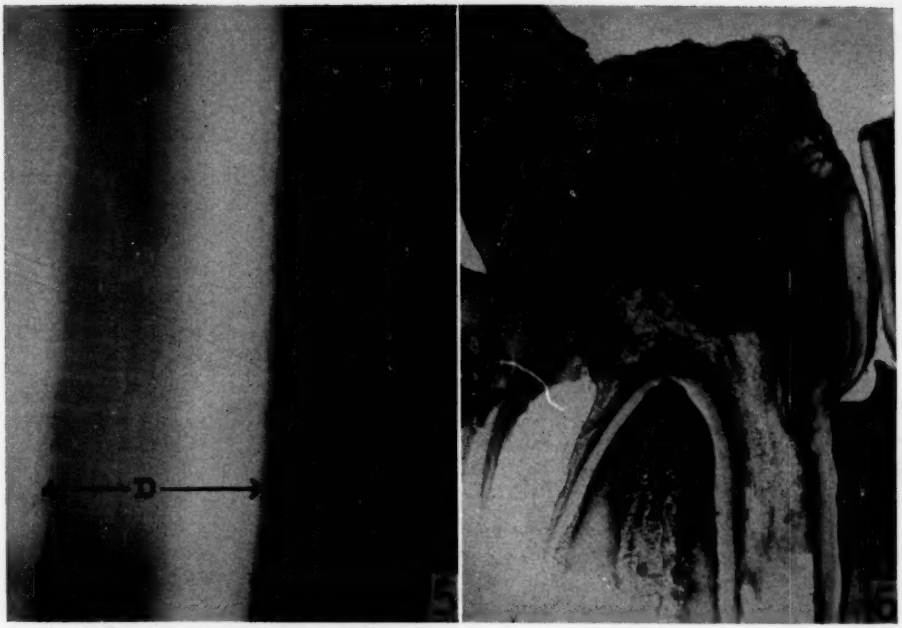

FIG. 5.—Ground section of tooth of tetracycline-injected specimen showing dentin, *D*, and enamel, *E*, photographed in ultraviolet light. Note that fluorescence in dentin is much more pronounced than that shown in enamel.

FIG. 6.—Upper second molar of rat injected with tetracycline. (Low-power, visible light.)

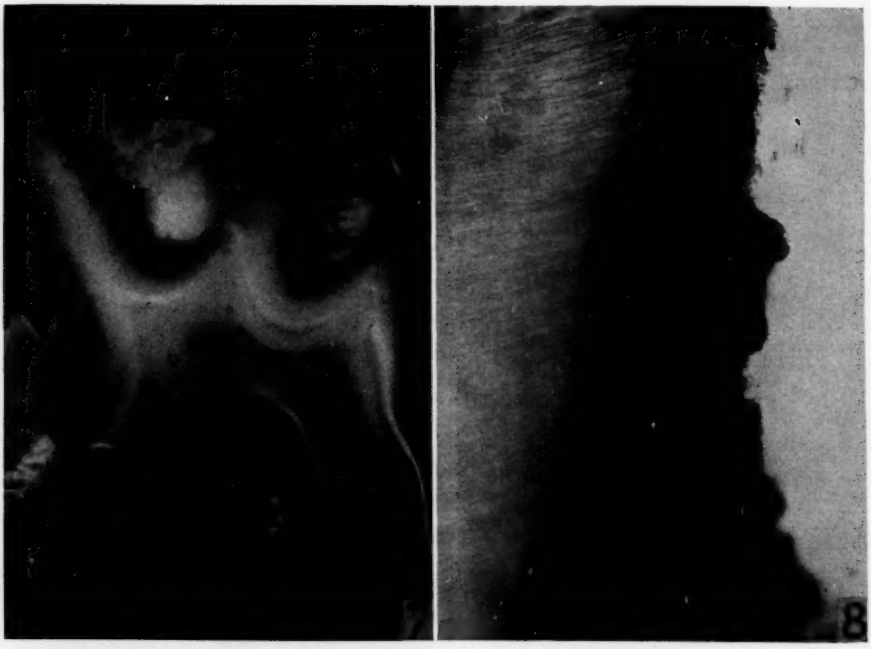

FIG. 7.—Same specimen as Fig. 6 photographed in ultraviolet light. Note brilliant fluorescent areas in dentin, indicative of tetracycline incorporation.

FIG. 8.—Dentin of portion of root of specimen shown in Figs. 6 and 7. Note banding of dentin photographed in visible light.

RESULTS

Incisors.—The incisors of the rats injected with tetracycline responded to this treatment in a characteristic manner. In the first place, the fluorophore was incorporated into the calcified itssues which were laid down during the experimental period (Figs. 1 and 2). Second, each daily injection was clearly represented in the developing dentin and enamel by a definite band corresponding to each injection of the drug administered (Figs. 3, 4, and 5). Furthermore, administration of 5 mg. of the drug also resulted in the deposition of dentin and enamel which was calcified to a lesser degree than normal

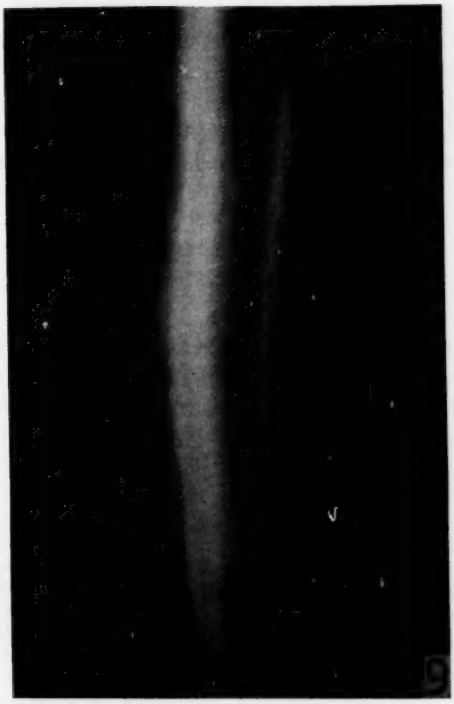

FIG. 9.—Portion of dentin shown in Fig. 8, photographed in ultraviolet light. Note prominent fluorescent bands in root.

dentin and enamel, as shown by the striations in these tissues. In addition, tetracycline was also incorporated into the growing bone, illustrated by the fluorescence of the alveolus and interradicular bone illustrated in Figures 2 and 7, respectively.

Molars.—Examination of the molars of the animals injected with tetracycline indicated that they responded in much the same manner as did the incisors. This is well shown in Figures 6 and 7, illustrating a representative molar photographed in visible and ultraviolet light, respectively. Figures 7 and 9 illustrate the presence of the fluorophore in this permanent tooth. In addition to the incorporation of the fluorophore, the experimental treatment also resulted in a modification (inhibition) of calcification, as shown in Figure 8.

DISCUSSION

In the studies of Milch *et al.*[5] it was shown that tetracycline (or a fluorophore of tetracycline) was incorporated in the bones of young adult animals and retained for a considerable period. In our previous studies[6–7] we demonstrated that administration of tetracycline to embryos resulted in the incorporation of a fluorophore in the developing skeletons. Administration of higher levels of the drug resulted in partial or complete inhibition of mineralization. It has also been reported[8] that prolonged administration of tetracycline during the gestation period of rats resulted in the production of several skeletal anomalies in the newborn animals.

With reference to the growing teeth, our present experiments have demonstrated that administration of tetracycline results in the incorporation and retention of a fluorophore and a partial inhibition of mineralization in the increments of enamel and dentin which are formed during the period of the experimental treatment.

We suggest, on the basis of the observations referred to above, that long-term studies devoted to an appraisal of tetracycline therapy in human children would be extremely desirable.

SUMMARY

Tetracycline hydrochloride was administered to young rats in the amount of 5 mg. for each of 5 successive days.

Examination of the teeth following this treatment revealed the presence of fluorescent bands in the dentin and enamel in the incisors and molars corresponding to the 5 injections of the drug administered. In addition to the incorporation and retention of tetracycline as shown by the presence of the fluorophore, inhibition of calcification also occurred.

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The Effect of Cow's Milk on Dental Caries in the Rat

SAMUEL DREIZEN, JO G. DREIZEN, and ROBERT E. STONE

Department of Nutrition and Metabolism, Northwestern University Medical School, Chicago, Illinois.

Studies by McClure and Folk,[1, 2] McClure,[3] and Bavetta and McClure[4] suggest that dry skim milk prepared by either the roller or the spray process is cariogenic for the white rat. The dental caries-producing potential of the milks tested paralleled the severity of the commercial heat treatment employed in the drying procedures.[3] Bavetta and McClure[4] attributed the cariogenicity of diets containing heat-processed skim-milk powder to a critical deficiency of lysine. Recently, however, Mauron, Kinkel, and Cremer[5] reported that the heating of dry whole milk affects neither the incidence nor the degree of dental caries in the rat and that there is no significant relationship between the content of utilizable lysine and the cariogenic potency of milk-containing diets.

In each of the afore-mentioned investigations, dry milk powder comprised from 35 to 40 per cent of the total experimental diet. Data pertaining to the cariogenicity of heat-treated cow's milk in rats fed milk alone are lacking. To permit a more comprehensive evaluation of the relationship between dry cow's milk and rat dental caries, a comparison has been made of the dental caries incidence in rats restricted to diets containing 0, 39, and 100 per cent non-fat dry milk.

MATERIALS AND METHODS

Three randomly selected groups, each containing 30 Sprague-Dawley male albino rats, were given the following diets and tap water ad libitum beginning at 5 weeks of age. Group I received degerminated white corn meal, 35 per cent; white wheat flour, 28 per cent; granulated sugar, 19 per cent; and prime steam lard, 18 per cent. The corn meal and wheat flour were enriched with niacin, thiamin, riboflavin, and iron. The ingredients were mixed with sufficient water to make a thick dough, rolled into thin layers, baked until dry, and offered to the animals daily in a finely ground form. Group II was fed the corn meal, wheat flour, sugar, and lard mixture 61 per cent, and non-fat dry milk 39 per cent. The nutrient value of the milk powder as determined by direct chemical analysis was carbohydrate, 51.4 per cent; protein, 34.2 per cent; fat, 0.65 per cent; fiber, 0.26 per cent; ash, 7.58 per cent; and moisture, 5.91 per cent. The lysine content was 3.20 gm/100 gm of milk powder. Group III was given only the non-fat dry milk.

The rats were housed in individual screen-bottom cages and kept in an air-condi-

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tioned room maintained at approximately 23° C. They were weighed each week and subjected to the experimental regimens until they expired. At necropsy, the jaws were removed, stripped of all soft tissue, and kept under water until the molar teeth were examined for caries. The teeth were examined while moist under low-power magnification (12×) in the manner described by McClure,[3] using a dissecting microscope and reflected light. The maxillae and mandibles were sectioned mid-sagittally and the molar teeth X-rayed. The X-rays were scrutinized under low-power magnification (12×) for evidence of interproximal decay. The data were then subjected to statistical analysis.

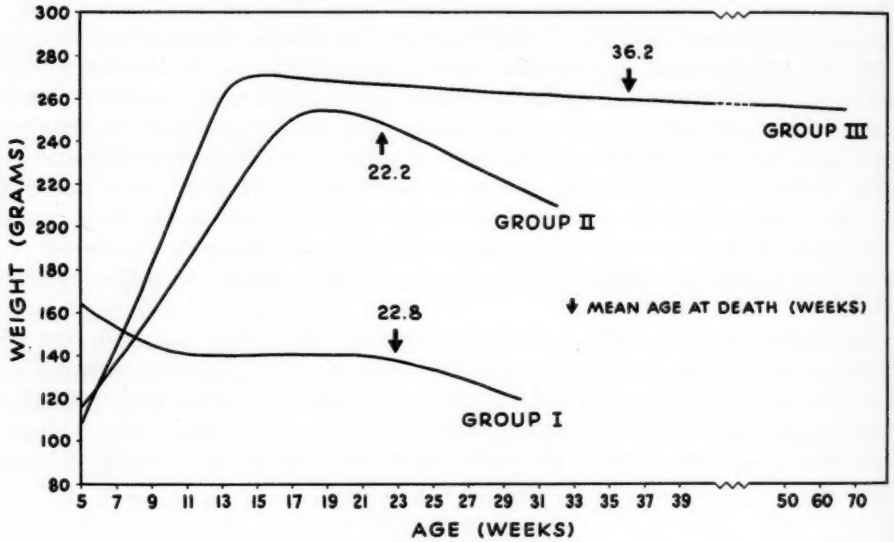

FIG. 1.—Growth and longevity of rats on experimental diets

RESULTS

A comparison of the mean patterns of growth and longevity of the rats on the various experimental diets is presented in Figure 1. None of the diets was conducive to optimum rat growth. The protein-deficient corn meal, wheat flour, sugar, and lard mixture failed to support even minimal growth. Animals confined to this diet (Group I) expired between 20 and 30 weeks of age. The mean age at death of 22.8 weeks was almost identical with that of Group II (22.2 weeks), the diet of which contained 39 per cent non-fat dry milk. Rats receiving the cereal, sugar, and lard diet with 39 per cent non-fat dry milk grew steadily until the eighteenth week of age, undergoing a progressive weight loss thereafter and expiring between 18 and 32 weeks of age. The 100 per cent non-fat dry milk fed the rats in Group III promoted relatively good growth. The mean age at death in this group was 36.2 weeks, with a range of from 17 to 69 weeks. Thus the rats were exposed to the test diets from a minimum of 84 days to a maximum of 448 days. The differences between the mean ages at death of Groups I and II and that of Group III were statistically significant ($p = < 0.01$).

As shown in Table 1, carious molar teeth were found in 20 (66.7 per cent), 23 (76.7 per cent), and 2 (6.7 per cent) of the rats in Groups I, II, and III, respectively. Of the total of 360 molar teeth in each group, 43 (11.9 per cent) of those in Group I, 64 (17.8 per cent) in Group II, and 2 (0.6 per cent) in Group III developed caries during the experimental period. The mean numbers of lesions per rat were 1.43 for Group I, 2.13 for Group II, and 0.07 for Group III. Whereas the difference between the means of Groups I and II was not statistically significant ($p = > 0.05$), that between Groups I and III and between Groups II and III was highly significant ($p = < 0.01$).

Each of the carious lesions detectable microscopically or roentgenographically was of the occlusal pit and fissure type. No smooth-surface lesions were found in this series. The frequency of involvement was slightly higher in the upper than in the lower molar teeth in each group. The degree of carious destruction was noticeably greater in the rats fed the corn meal, wheat flour, sugar, and lard mixture enriched with 39 per cent non-fat dry milk (Group II) than in those given the identical diet without the added milk (Group I). In contrast, the two lesions found in the animals given 100 per cent non-fat dry milk (Group III) were each confined to the distal pit of the upper left third molar.

TABLE 1

COMPARISON OF DENTAL CARIES PREVALENCE IN MOLAR TEETH OF RATS FED 0, 39, AND 100 PER CENT NON-FAT DRY MILK

Group	Milk in Diet (Per Cent)	No. of Rats	Caries Prevalence (Rats)		Caries Distribution (Teeth)			
			No.	Per Cent	Upper	Lower	Total	Mean
I.....	0	30	20	66.7	28	15	43	1.43
II.....	39	30	23	76.7	34	30	64	2.13
III.....	100	30	2	6.7	2	0	2	0.07

DISCUSSION

The present findings clearly demonstrate that non-fat dry cow's milk is not cariogenic for the white rat when constituting the sole source of nutriment. The almost complete absence of dental caries in rats fed only non-fat dry milk cannot be ascribed to any known specific anticariogenic factor inherent in this food. The cariogenicity of the corn meal, wheat flour, sugar, and lard mixture used in this study was not changed significantly when dry non-fat milk was substituted in this diet to the extent of 39 per cent by weight.

Bavetta and McClure[4] noted that the protein content of the diet may be an important factor in the initiation of rat dental caries. Rats given a diet containing 13 per cent casein developed a high incidence of severe caries, whereas raising the casein concentration to 24 per cent resulted in very limited caries formation. Although the present studies support these observations (the non-fat milk contained approximately 28 per cent casein), the mechanism of the protective action of high concentrations of casein against rat dental caries remains obscure.

Reports by McClure and Folk,[1, 2] McClure,[3] and Bavetta and McClure,[4] and by Stephan,[6] and Stephan, Harris, and Fitzgerald[7] of the induction of dental caries in rats by skim-milk-powder diets stress the frequency of appearance of smooth-surface lesions. Only pit and fissure type occlusal caries were encountered under the experimental conditions described herein. The lack of smooth-surface decay in the present series may be related to the relatively high fat content of the protein-deficient corn meal, wheat flour, sugar, and lard cariogenic diet, a characteristic which distinguished it from the low-fat diets used by McClure[1–4] and Stephan.[6, 7]

SUMMARY

The role of non-fat dry cow's milk in experimental rat caries was determined from feeding tests in which the milk comprised 39 and 100 per cent by weight of the total diet. At the 100 per cent level, the milk was non-cariogenic for the rat. At the 39 per cent level, the milk had no appreciable effect on the cariogenicity of a high-carbohydrate, high-fat, low-protein diet.

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The Dental Tissues of Wild and Laboratory-raised Hibernating and Non-hibernating 13-lined Ground Squirrels

ROBERT L. RICHARDSON, ALTON K. FISHER, and G. EDGAR FOLK, JR.

Departments of Bacteriology, Stomatology, and Physiology, State University of Iowa, Iowa City, Iowa

The 13-lined ground squirrel, *Cittelus tridecemlineatus,* is a valuable animal for laboratory investigations because it hibernates at low room temperatures. Although today it is a common laboratory animal, few reports are available pertaining to its dental tissues. Schour[1] described the changes seen in the incisor of the 13-lined ground squirrel following gonadectomy, and Mayer and Bernick[2, 3] studied the periodontal tissues and caries incidence of the Arctic ground squirrel.

The 13-lined ground squirrel has a dental formula of incisors 2/2, premolars 4/2, and molars 6/6. The anatomy of the animal's posterior teeth is similar to the corresponding posterior teeth of human beings.

This report is one of several originating in the laboratories of G. E. Folk, Jr., and concerned with the effects of hibernation on metabolic and physiologic activities of the ground squirrel.[4]

MATERIALS AND METHODS

In this study three groups of ground squirrels were used. Each group represented a different environmental history. One group, referred to as the "non-hibernating ground squirrels," was comprised of 14 ground squirrels that were born and raised in a laboratory maintained at 26° C. At 36 months of age they were sacrificed and the oral tissued examined. The second group, referred to as the "hibernating ground squirrels," was comprised of 11 animals born and raised under conditions similar to those of the non-hibernating ground squirrels, except that they were exposed to a room temperature of 6° ± 2° C. for 4 months during each of three winters. This provided 12 months of hibernation during their 36 months of life. The dental examinations for this group were made within 6 hours following removal of the animals from their terminal period of hibernation. The diet of the non-hibernating and hibernating squirrels consisted of Rockland rat pellets, weekly rations of mixed grain, and water ad libitum; each animal was isolated at all times in a Bussey cage. Additional information concerning the care and maintenance of these animals is available.[4, 5] The third group, referred to as the "wild ground squirrels," was composed of 42 ground squirrels, of adult size and unknown age, that were captured in Iowa.

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Soon after the animals were sacrificed, a gross examination of the oral tissues was made on all ground squirrels, with the aid of a dissecting microscope. Special attention was given to missing teeth, mobility of teeth, health of gingivae, occlusal wear, carious lesions, length and color of incisors, and amount of dental plaque, stain, and calculi. To supplement the written records, photographs and roentgenograms were made frequently.

A bacteriological examination was made of oral scrapings from five non-hibernating and seven hibernating animals. Early during the gross examination, soft debris was scraped from the proximal surfaces of molars and inoculated on various bacteriologic media. Inoculated plates of blood agar and lactobacillus selective medium were incubated anaerobically at 37° C. for 48 hours; another inoculated blood-agar plate and a nutrient agar plate were incubated in air at 10° C. for 7 days. Inoculated plates of eosin methylene blue and Chapman Stone medium were incubated aerobically at 37° for 48 hours. The genus identification was established for the predominating microorganisms cultured.

A histologic analysis was made on the jaws of ten non-hibernating, ten hibernating, and eight wild ground squirrels. Mesiodistal (or anterior-posterior) paraffin sections were cut from the upper and lower jaws so as to show the tissues extending from the diastema between the incisors and premolars to the posterior limits of the retromolar space. Sections were stained with hematoxylin and eosin and for demonstration of acid mucopolysaccharides. A total of 22 periodontal tissue areas was available from each animal for examination. Pathologic alterations were graded and were reported as that percentage of the total periodontal area in each group of animals that showed pathologic changes. A more complete report of the histologic analysis than that presented here is available.[6]

RESULTS

The periodontal tissues of the laboratory-raised, non-hibernating, and hibernating ground squirrels exhibited marked pathologic alternations, and the diseased oral tissues of these ground squirrels contrasted sharply with the healthy periodontium observed in the wild ground squirrels.

The pathologic changes observed in the dental tissues of the laboratory-raised, non-hibernating, and hibernating ground squirrels were similar in many respects, but three differences were apparent. One difference was that severe inflammatory reactions were observed twice as frequently in the lower jaws of the hibernating ground squirrels as in the lower jaws of the non-hibernating animals. A second difference was that ulceration of the interdental papillae, pathologic gingival pocket formation, and hyperplasia of the gingival crevicular epithelium occurred approximately twice as frequently in the lower jaws of the hibernating animals as in the lower jaws of the non-hibernating ground squirrels. The third difference noted was that significantly more crowns were missing from the lower jaws of the non-hibernating than from the lower jaws of the hibernating ground squirrels. In all three groups the periodontal tissues of the lower jaws showed more frequent and severe pathologic manifestations than that observed in the upper jaws.

The number of posterior teeth present in the three groups varied greatly. Some 75 per cent of the crowns of the lower posterior teeth were missing in the non-hibernating

ground squirrels, and 52 per cent of the crowns of the lower posterior teeth were missing in the hibernating animals. The difference between these percentages is significant ($P = 0.02$). Only 6 per cent of the crowns of upper posterior teeth were missing from non-hibernating and hibernating animals. All teeth in all wild ground squirrels were present. While the crowns of teeth were missing, their roots were usually retained. Severe chronic inflammatory reactions were frequently observed around retained roots and in the exposed dental pulps.

Debris, which consisted primarily of wads of hair, was frequently found impacted in the interproximal spaces of the upper and lower posterior teeth of the hibernating and non-hibernating ground squirrels, but was not observed in any of the wild ground squirrels (Fig. 1, *A, B, C, D*). The most extensive impactions were found in the upper jaws in the interproximal and palatal-gingival areas. The impacted debris was probably the irritant most responsible for causing the pathologic changes which resulted in loss of hard and soft supporting tissues. Where there was considerable impaction of debris, half or more of the molar roots were frequently exposed (Fig. 1, *C, D*). Teeth so involved exhibited increased mobility.

Plaque often covered the posterior teeth of the non-hibernating and hibernating ground squirrels. Reduced masticatory function, imposed by the absence of many lower teeth, presumably contributed to the accumulation of the soft material. The occlusal surfaces of the wild ground squirrels were frequently so free of plaque that they appeared polished. Occlusal abrasion was slightly greater in the wild ground squirrels than in the non-hibernating and hibernating animals.

Dental calculus was found on the buccal surfaces of upper premolars and first molars of 35 per cent of all laboratory-raised ground squirrels. These deposits were confined primarily to the anatomical crowns. Calculus was not observed in any of the wild ground squirrels. Around the cervices of the wild ground squirrels' teeth, a brown, leathery deposit was often observed (Fig. 1, *A*). This material was possibly derived from grass seeds and grains, since the cheek pouches of the wild ground squirrels often contained seeds.

Slight inflammatory changes were observed in 20 per cent of the periodontal tissue areas of the wild ground squirrels and in 40 per cent of the periodontal areas of the non-hibernating and hibernating animals. Extensive inflammatory changes and ulcerated interdental papillae were observed in approximately 25 per cent of the periodontal areas in the upper jaws of both non-hibernating and hibernating ground squirrels and in 25 per cent of the periodontal areas of the lower jaws of the non-hibernating animals. Some 50 per cent of the periodontal tissue areas in the lower jaws of the hibernating ground squirrels showed extensive inflammatory changes and ulcerated papillae. In the wild ground squirrels, extensive inflammatory changes and ulcerated interdental papillae were noted in 8 per cent of the periodontal areas in the lower jaws and rarely in the upper jaws.

Hyperplastic crevicular epithelium and pathologic gingival pockets were observed in the laboratory-raised, non-hibernating, and hibernating ground squirrels but were not seen in the wild ground squirrels. These two pathologic changes were noted in approximately 20 per cent of the periodontal areas in the lower jaws of the non-hibernating squirrels and in about 40 per cent of the corresponding areas in the hibernating group. In both groups these changes were observed approximately twice as often in the lower

jaws as in the upper jaws. The occurrence of pathologic gingival pockets and hyperplastic epithelium corresponded reasonably well with the inflammation and ulceration of the interdental papillae, as described above. The resulting structure of the periodontal bone reflected the severity of the inflammatory changes in the immediate environment, but no significant non-inflammatory osteoporosis was observed.

All incisors in all three groups were present. There were no significant differences in upper incisor lengths or in lower incisor lengths for the three groups. Measured from the gingival margin to the incisal edge in the mid-line, the mean lengths of the upper incisors were 2.4 mm. (S.D. 0.4) for the non-hibernating squirrels, 2.1 mm. (S.D. 0.4) for the hibernating squirrels, and 2.5 mm. (S.D. 0.5) for the wild ground squirrels. The mean lengths of the lower incisors were 6.9 mm. (S.D. 0.9) for the non-hibernating

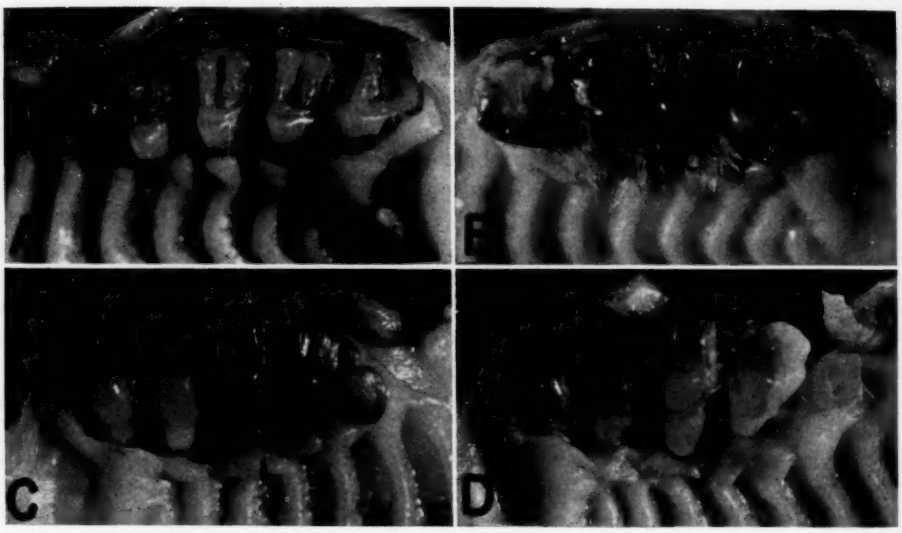

FIG. 1.—*A*, Palatal view of upper left jaw of a wild ground squirrel showing a healthy periodontium, typical of those observed in this group of animals. Notice that the gingiva hugs the teeth closely and that it completely occupies the interproximal regions. Hair is not present between the teeth. The teeth are in good alignment, and the occlusal surfaces are free of plaque, although there is a little stained debris in a few grooves. The brown-stained deposit, which is most prevalent around the cervices of the teeth, was observed frequently in the wild ground squirrels. It is possible that the deposit and stain were acquired largely from seeds, for they were frequently found in the cheek pouches of the wild ground squirrels. The teeth shown are, from left to right, the left first premolar, second premolar, first molar, second molar, and third molar. *B*, Palatal view of upper left jaw of a laboratory-raised non-hibernating ground squirrel showing an unhealthy periodontium, typical of those observed in this group of animals. Notice the impacted hair in the interproximal spaces. An extensive mass of hair between the first and second premolars has caused a marked mesial malpositioning of the first premolar. Calculus is present on the buccal of the first premolar. A moderately thick film of plaque covers the occlusal surface of all teeth. The oral conditions observed in this illustration not only are typical of those of the laboratory-raised non-hibernating ground squirrels but are also representative of the conditions observed in the laboratory-raised hibernating animals, since the gross appearance of the periodontal tissues of the two groups was similar. *C*, Palatal view of upper right jaw of a laboratory-raised hibernating ground squirrel. Impacted hair was removed from the interproximal areas, so that the recessed supporting tissues and the exposed roots might be better seen. *D*, Palatal view of upper right jaw of a laboratory-raised non-hibernating ground squirrel. Hair was removed from between the teeth to facilitate demonstrating the exposed roots, the loss of supporting tissue, and the drifting of the teeth which occurred. A considerable amount of plaque is present on the occlusal surfaces and exposed roots.

squirrels, 6.3 mm. (S.D. 0.9) for the hibernating animals, and 6.5 mm. (S.D. 0.8) for the wild animals.

In all three groups of animals only one carious lesion was observed, and it occurred adjacent to a fractured cusp in one of the wild ground squirrels.

No difference was observed between the oral bacterial floras of the non-hibernating and hibernating ground squirrels. Micrococci, alpha and non-hemolytic streptococci were isolated from nearly all specimens. Species of *Corynebacterium, Flavobacterium, Sarcina,* and *Gaffkya* were the predominating organisms cultured from the plates incubated at 10° C.

DISCUSSION

Few studies have compared the oral tissues of laboratory-raised and wild animals. The only study to come to our attention that mentions such a comparison is that of Mayer and Bernick,[3] who studied the incidence of caries in wild and laboratory-raised Arctic ground squirrels. Our study was significant because it showed that normal laboratory procedures of care and maintenance, which included a diet presumed to furnish all the animals' nutritional needs, did not promote or provide a healthy periodontium for the ground squirrel, while conditions of nature did promote a healthy periodontium. The periodontal disease did not keep the laboratory-raised animals from maintaining their original adult level of body weight (mean weight 1955, 163.7 gm.; 1957, 164.7 gm.).

Our study failed to indicate unequivocally that hibernation per se had either an adverse or a protective effect on the dental tissues of the ground squirrel. The observation that significantly more crowns were present in the lower jaws of the hibernating animals than in the non-hibernating ground squirrels would seem to indicate that some factors associated with hibernation enabled the hibernating ground squirrels to retain their lower teeth longer than was the case with the non-hibernating animals. On the other hand, an indication that hibernation had an adverse affect on the periodontal tissues is suggested in the observation that severe inflammatory reactions, crevicular epithelial hyperplasia, pocket formation, and ulceration of the interdental papillae occurred twice as frequently in the lower jaws of the hibernating squirrels as in the non-hibernating animals. These differences were not noted in the upper jaws, however.

It is difficult to evaluate how important the impacted hair was in contributing to the periodontal disease observed in the caged animals. We believe it was of considerable importance and suggest that, had the laboratory-raised squirrels been in a natural environment with less tendency to lick themselves, it is likely that the severity of the periodontal disease would have been considerably reduced.

Baer and Lieberman[7] observed hair impaction associated with loss of supporting tissues in 5 of 6 strains of mice. In their study the most severe impactions occurred in 2 strains that exhibited alopecia. Alopecia was not observed in the ground squirrel we studied. Baer and Lieberman also noted that periodontal disease was more severe in female than male mice and that the more severe lesions occurred in the upper jaws. In our study the periodontal disease affected both sexes approximately equally, and the more severe periodontal changes were observed in the lower jaws.

Mayer and Bernick[2] observed disruption of normal dentinogenesis, osteoporosis, and rather severe tissue changes in the periodontium of Arctic ground squirrels after 1

jaws as in the upper jaws. The occurrence of pathologic gingival pockets and hyperplastic epithelium corresponded reasonably well with the inflammation and ulceration of the interdental papillae, as described above. The resulting structure of the periodontal bone reflected the severity of the inflammatory changes in the immediate environment, but no significant non-inflammatory osteoporosis was observed.

All incisors in all three groups were present. There were no significant differences in upper incisor lengths or in lower incisor lengths for the three groups. Measured from the gingival margin to the incisal edge in the mid-line, the mean lengths of the upper incisors were 2.4 mm. (S.D. 0.4) for the non-hibernating squirrels, 2.1 mm. (S.D. 0.4) for the hibernating squirrels, and 2.5 mm. (S.D. 0.5) for the wild ground squirrels. The mean lengths of the lower incisors were 6.9 mm. (S.D. 0.9) for the non-hibernating

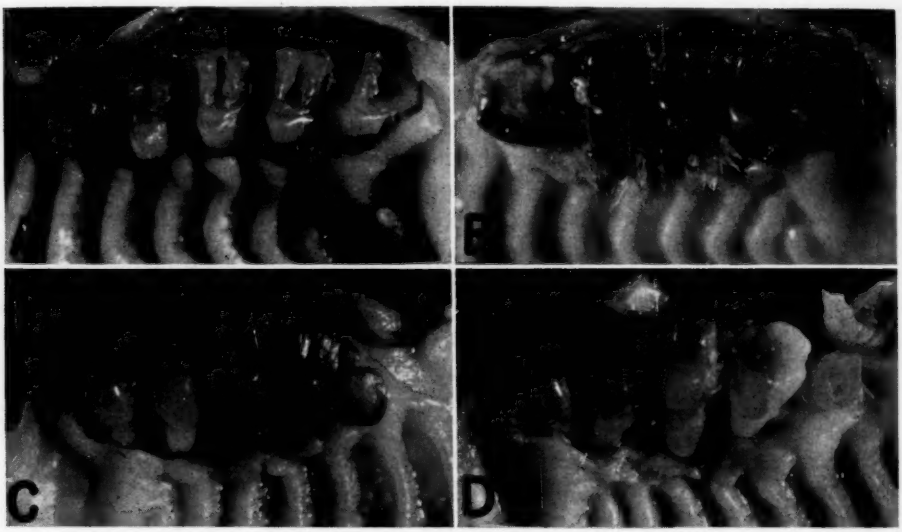

FIG. 1.—*A,* Palatal view of upper left jaw of a wild ground squirrel showing a healthy periodontium, typical of those observed in this group of animals. Notice that the gingiva hugs the teeth closely and that it completely occupies the interproximal regions. Hair is not present between the teeth. The teeth are in good alignment, and the occlusal surfaces are free of plaque, although there is a little stained debris in a few grooves. The brown-stained deposit, which is most prevalent around the cervices of the teeth, was observed frequently in the wild ground squirrels. It is possible that the deposit and stain were acquired largely from seeds, for they were frequently found in the cheek pouches of the wild ground squirrels. The teeth shown are, from left to right, the left first premolar, second premolar, first molar, second molar, and third molar. *B,* Palatal view of upper left jaw of a laboratory-raised non-hibernating ground squirrel showing an unhealthy periodontium, typical of those observed in this group of animals. Notice the impacted hair in the interproximal spaces. An extensive mass of hair between the first and second premolars has caused a marked mesial malpositioning of the first premolar. Calculus is present on the buccal of the first premolar. A moderately thick film of plaque covers the occlusal surface of all teeth. The oral conditions observed in this illustration not only are typical of those of the laboratory-raised non-hibernating ground squirrels but are also representative of the conditions observed in the laboratory-raised hibernating animals, since the gross appearance of the periodontal tissues of the two groups was similar. *C,* Palatal view of upper right jaw of a laboratory-raised hibernating ground squirrel. Impacted hair was removed from the interproximal areas, so that the recessed supporting tissues and the exposed roots might be better seen. *D,* Palatal view of upper right jaw of a laboratory-raised non-hibernating ground squirrel. Hair was removed from between the teeth to facilitate demonstrating the exposed roots, the loss of supporting tissue, and the drifting of the teeth which occurred. A considerable amount of plaque is present on the occlusal surfaces and exposed roots.

squirrels, 6.3 mm. (S.D. 0.9) for the hibernating animals, and 6.5 mm. (S.D. 0.8) for the wild animals.

In all three groups of animals only one carious lesion was observed, and it occurred adjacent to a fractured cusp in one of the wild ground squirrels.

No difference was observed between the oral bacterial floras of the non-hibernating and hibernating ground squirrels. Micrococci, alpha and non-hemolytic streptococci were isolated from nearly all specimens. Species of *Corynebacterium, Flavobacterium, Sarcina,* and *Gaffkya* were the predominating organisms cultured from the plates incubated at 10° C.

DISCUSSION

Few studies have compared the oral tissues of laboratory-raised and wild animals. The only study to come to our attention that mentions such a comparison is that of Mayer and Bernick,[3] who studied the incidence of caries in wild and laboratory-raised Arctic ground squirrels. Our study was significant because it showed that normal laboratory procedures of care and maintenance, which included a diet presumed to furnish all the animals' nutritional needs, did not promote or provide a healthy periodontium for the ground squirrel, while conditions of nature did promote a healthy periodontium. The periodontal disease did not keep the laboratory-raised animals from maintaining their original adult level of body weight (mean weight 1955, 163.7 gm.; 1957, 164.7 gm.).

Our study failed to indicate unequivocally that hibernation per se had either an adverse or a protective effect on the dental tissues of the ground squirrel. The observation that significantly more crowns were present in the lower jaws of the hibernating animals than in the non-hibernating ground squirrels would seem to indicate that some factors associated with hibernation enabled the hibernating ground squirrels to retain their lower teeth longer than was the case with the non-hibernating animals. On the other hand, an indication that hibernation had an adverse affect on the periodontal tissues is suggested in the observation that severe inflammatory reactions, crevicular epithelial hyperplasia, pocket formation, and ulceration of the interdental papillae occurred twice as frequently in the lower jaws of the hibernating squirrels as in the non-hibernating animals. These differences were not noted in the upper jaws, however.

It is difficult to evaluate how important the impacted hair was in contributing to the periodontal disease observed in the caged animals. We believe it was of considerable importance and suggest that, had the laboratory-raised squirrels been in a natural environment with less tendency to lick themselves, it is likely that the severity of the periodontal disease would have been considerably reduced.

Baer and Lieberman[7] observed hair impaction associated with loss of supporting tissues in 5 of 6 strains of mice. In their study the most severe impactions occurred in 2 strains that exhibited alopecia. Alopecia was not observed in the ground squirrel we studied. Baer and Lieberman also noted that periodontal disease was more severe in female than male mice and that the more severe lesions occurred in the upper jaws. In our study the periodontal disease affected both sexes approximately equally, and the more severe periodontal changes were observed in the lower jaws.

Mayer and Bernick[2] observed disruption of normal dentinogenesis, osteoporosis, and rather severe tissue changes in the periodontium of Arctic ground squirrels after 1

month of hibernation. The periodontal changes they observed included pocket formation, which occasionally extended to the lower half of the roots, calculus formation on the cementum, loss of bone to a degree which frequently exposed the bifurcation of roots, and occasionally, apical abscesses. These severe changes occurred in only 1 month of hibernation. We would estimate that the pathologic changes observed in our group of hibernating squirrels were about equal in severity to those Mayer and Bernick observed, but the changes observed in our hibernating group were acquired over 36 months, including 12 months of hibernation, rather than 1 month. It is significant, too, that in our study these periodontal changes were not confined to hibernating ground squirrels but were also found in the non-hibernating animals.

In another study, Mayer and Bernick[3] observed carious lesions frequently in the posterior teeth of the Arctic ground squirrels kept in captivity for extended periods, but they did not observe dental caries in wild Arctic ground squirrels. They thought that conditions of captivity, including diet, were responsible for the development of the carious lesions. In our study, only one carious lesion was observed in the three groups of ground squirrels. Apparently our ground squirrels were fed a much less cariogenic diet than the one that Mayer and Bernick fed their Arctic ground squirrels.

The observation that there were no significant differences in incisor lengths deserves comment. Since the hibernating group of ground squirrels spent one-third of their lives in hibernation, it might be supposed that the interruption of the normal attrition rate imposed by hibernation would be recognized by an increase in incisor length. If this did occur, then the attrition rate during the non-hibernating period was sufficient to compensate for the length gained during hibernation. It seems more probable that, since many metabolic processes are reduced during hibernation, those involved in incisor eruption were also reduced and that little eruption actually occurred during hibernation. The design of the experiment did not permit the eruption rates to be measured. It should be noted that drug-induced prolonged sleep increased the eruption rate of the rat's incisors.[8]

The bacteriologic analysis was undertaken to determine whether major differences existed between the oral bacterial floras of the non-hibernating and those of the hibernating ground squirrels. Since the major bacterial populations of the two groups were similar, it seems reasonable to suggest that the oral bacteria established during periods out of hibernation are retained through periods of hibernation. The possibility of quantitative changes in bacterial populations remains to be investigated.

SUMMARY

Periodontal tissue changes were observed much more frequently in non-hibernating and hibernating ground squirrels maintained in the laboratory for 36 months than they were in wild ground squirrels captured in their natural environment.

The principal changes observed in laboratory-raised ground squirrels included extensive impactions of debris between the teeth, loss of approximately 50 per cent of the crowns of the lower posterior teeth, and severe inflammatory reactions around retained roots.

Hibernating (laboratory-raised) ground squirrels showed pathologic disturbances similar to those observed in the non-hibernating animals, except that extensive inflammatory reactions, crevicular epithelial hyperplasia, pocket formation, and ulcerated

papillae were observed approximately twice as frequently in the lower jaws of hibernating squirrels as in the non-hibernating animals.

Significantly more crowns were missing from the lower jaws of the non-hibernating animals than from the lower jaws of the hibernating ground squirrels.

Pathologic changes in the periodontium were observed more frequently in the lower jaws than in the upper jaws in all three groups of ground squirrels, and they were observed with equal frequency in both sexes.

No significant differences were observed between the incisor lengths of non-hibernating, hibernating, and wild ground squirrels.

Only one carious lesion was observed in all three groups of ground squirrels.

The micro-organisms comprising the oral floras of non-hibernating and hibernating ground squirrels were similar.

This study failed to provide unequivocal evidence that hibernation per se had either an adverse or a protective affect on the dental tissues of the ground squirrels.

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Meperidine and Promethazine Hydrochloride for Handicapped Patients

MANUEL M. ALBUM
Medical Arts Building, Jenkintown, Pennsylvania

Premedication in the dentistry of retarded, emotionally disturbed, or disabled children (cerebral palsy) presents special problems. There are several goals which are on a different level from those sought in normal children. Many times it is impossible to perform routine chores such as examination and cleaning in these patients. Premedication, as an adjunct in treatment, should, by sedating and tranquilizing the patient, induce co-operation and aid in obtunding him to pain. The usual pediatric dosage recommendations are totally unable to achieve these goals in these children.

Promethazine* has been reported to be a useful sedative, preanesthetic medication by itself,[1, 2] to have a definite antiemetic effect,[3] and to be an effective supplement to anesthesia.[4] It has been used in dentistry for control of postextraction edema,[5] pain and trismus,[6] and for pre-operative sedation.[7] Steubner and Sadove[8] established the sedative dose for children in preoperative use at 0.5 mg/lb of body weight. Promethazine has been shown not to be depress respiration in therapeutic doses.[9]

Meperidine is a potent narcotic agent with sedative and depressant effects. Extensive studies have indicated its respiratory depressant effects to be as great as 10 mg. of morphine sulfate at a 75-mg. dosage.[10]

The concomitant use of promethazine meperidine has led to studies on the combination of these drugs† which indicate that equivalent sedative activity can be achieved by replacing half the meperidine with promethazine.[11] At the same time, the respiratory depression is not increased by promethazine,[12] so that the margin of safety is greatly increased. The combination has been used successfully in preoperative dentistry.[13]

EXPERIMENTAL METHODS

The total study population was 71. Fifty were disturbed and retarded children who took part in the cross-over portion of the study, 5 were disturbed children who had to be studied as hospital outpatients, and 16 were normal office patients, included for comparative purposes. Their age range was from six to eighteen, with an average age of about thirteen.

The drugs included in the study were meperidine at 50 mg/cc, a combination of meperidine and promethazine, 25 mg of each drug/cc, and a saline placebo. The

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* Promethazine hydrochloride is available as Phenergan®, Wyeth Laboratories, Philadelphia, Pennsylvania.

† Available as Mepergan®, Wyeth Laboratories, Philadelphia, Pennsylvania.

placebo was included because it was of interest to determine whether a placebo effect existed in these children and also to insure that they would be able to select active medication from placebo.

The study utilized the double-blind cross-over technique in the handicapped children. These children were previously completely unamenable to dental work except in the hospital with very heavy premedication or total anesthesia. The subject drugs were put in randomly numbered multiple-dose vials, with one vial of each drug per "patient package." At each of three visits, the patients received one of the code medications from his own package, so that all three medications were given to each of fifty patients in random sequence. All the handicapped children were to receive 1 cc. of the first coded medication. If more medication was required, ½-cc. increments could be given to a total of 2 cc. This base-line dosage from the first visit would then be given for the other two coded drugs on the two successive visits. The clinical studies of the patients indicated that there would be a great variation from this group of patients to a group of normal patients. A true "between-drugs" comparison could be attained across fifty patients by using the inherent internalization of the cross-over technique.

The parameters to be used in evaluating these drugs were very simple. There was to be simple "Yes" or "No" scoring on the following questions: Was the patient cooperative? Was the patient amenable to work? Was the patient well sedated? Records were kept of side effects and the requirement for additional medication, so that some ancillary information could be obtained and to insure that repeat doses of medication did not interfere with the basic study design. All answers in the tables are those which occurred after the final dose of medication, if repeat dosages were needed. Since the first visit determined the total dosage, there was no variation in the same patient among the different medications in so far as dosage was concerned.

In addition, sixteen normal office patients were premedicated according to the routine recommendations and compared with this group, in order to determine whether the premedication requirements were as great as originally anticipated and whether the results were to be very far apart from those in the handicapped children.

Medication, when utilized for office procedures, was given between ½ and 1 full hour prior to the time of appointed dentistry. Additional medication was given if the patient obviously was not achieving the results desired. The normal office patients were given either meperidine or the meperidine-promethazine combination without any saline being used by the usual weight-dosage calculation. Their dosage varied from 0.25 to 1.0 cc.

RESULTS

The most striking finding early in the study was that the original assumption that 1 cc. of each medication would be an average for good results was completely false. Therefore, the more severely handicapped or emotionally disturbed children were graded clinically before receiving the medication and were given dosages of 1.5 or 2 cc. initially instead of the 1.0-cc. dose given to the cerebral palsy patient and the mildly disturbed patient. Figure 1 indicates that increase in the initial dosage decreased the incidence of repeat dosages. It did not increase the incidence of side effects, such as nausea, vomiting, syncope, and diaphoresis in these children. However, all patients who were originally started on a stated dosage, including the 1-cc. dosage, were continued

on that dose throughout the study, so that valid comparisons were maintained. Table 1 gives the over-all results of the premedication in these handicapped children. As can be seen from this table, the results from the promethazine-meperidine combination and meperidine alone were indistinguishable. One of the most striking aspects of the table is that the placebo effect, falling between 20 and 30 per cent, is in the same range as has been reported so frequently in literature on normal patients. It can also be seen that there was a tendency for sedation to be less completely achieved than was desirable.

There were five children (four mentally retarded and one mentally normal but handicapped by poliomyelitis) who could not be handled by this regimen in the office, despite very large doses. These were brought into the hospital for treatment on an outpatient basis.

The difference between the 16 "normal" and 50 handicapped office patients was

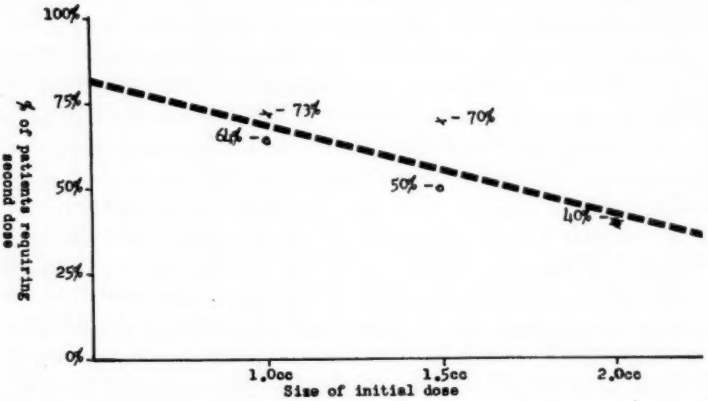

FIG. 1.—Correlation of size of initial dose and per cent of patients requiring second dose. O = meperidine promethazine; X = merperidine.

TABLE 1

PER CENT SUCCESSFUL EFFECTS WITH THREE DRUGS AT DIFFERENT DOSAGE LEVELS

		Co-operative	Amenable to Work	Well-sedated
1.0 cc. (30 pts.)	Meperidine-promethazine	80	80	80
	Meperidine	73	73	77
	Saline	27	30	20
1.5 cc. (10 pts.)	Meperidine-promethazine	80	80	70
	Meperidine	70	70	60
	Saline	20	20	10
2.0 cc. (10 pts.)	Meperidine-promethazine	60	60	60
	Meperidine	80	80	80
	Saline	30	30	30

striking. In usual dosages (0.5 mg. each of promethazine and meperidine per pound of body weight) the normal children were all successfully premedicated. Meperidine alone, 1 mg/lb of body weight, was successful in 13 of the 16 children. Obviously, then, the combination was very satisfactory in normal children at recommended dosages—more so than twice the amount of meperidine alone. It should also be stated here that another parameter was used in the normal office patients. In 14 of the 16 patients, the dentist made a choice of drug, which indicated his preference for the drug he believed to be most effective. In all 14 of these ballots, the selection was for the promethazine-meperidine combination, as the addition of the sedation and tranquilization was felt most desirable by these dentists.

Interesting findings were observed in the group of hospital patients under intravenous medication. Intravenous promethazine and meperidine were administered by a member of the anesthesia department. Oxygen and other emergency medication were available if needed. Ages of the patients ranged from eleven to sixteen years.

The patients received from 1 to 2 cc. of medication over a 2-minute interval. All five patients were tranquilized until dental procedures were attempted. One patient with poliomyelitis became difficult to handle when an attempt was made to administer a local anesthetic for the required dentistry. Association of previous experiences prevented anything from being done. Once outside the dental office, she became sleepy and relaxed. When returned to the dental environment, although completely relaxed, she became quite violent. Two mentally retarded patients reacted and were relaxed for dental procedures under intravenous medication. They were not sedated but tranquilized for the dentistry. Intravenous medication consisted of a total of $1\frac{1}{2}$ cc. Intravenous medication had no effect on the remaining two patients. These patients were discharged and told to return within several weeks. It was felt, since these patients were severely emotionally disturbed, that it would be better to relax them completely before the intravenous medication. Previous work has demonstrated the effectiveness of barbiturates in conduction with narcotic agents and has set the best dose in handicapped children at $2\frac{1}{2}$–3 grains of secobarbital.[14, 15] Therefore, three grains of secobarbital sodium* were given orally at home to these patients 1 hour prior to their dental appointment. When these patients came to the hospital, they were relaxed and ready for treatment. One patient was prepared for dentistry by the injection of 2 cc. of promethazine and meperidine intravenously.

One patient who had been given 3 grains of secobarbital sodium and 2 cc. intravenous promethazine and meperidine could be worked on but was stimulated by the dental bur when it was used in an unanesthetized area. At the third visit, the patient was medicated with the dose of the barbiturate and given 2 cc. of promethazine and meperidine during the first 2 minutes. An additional 0.6 cc. of the medication was given. It was then possible to give the patient local anesthesia, and the dentistry was performed without any problem. The patient had found his own threshold level of medication. When dentistry was performed on the unanesthetized side, the patient became stimulated and quite reactive. Five minutes after completion of the dental work, the patient again became reactive. Some mentally retarded patients apparently metabolize medication rapidly, since they are already on daily medication of some form or

* Secobarbital available as Seconal® from Eli Lilly & Co., Indianapolis, Indiana.

other. Fifteen minutes after the dental work was completed, the eyes were opened and reactive. Blood-pressure readings in this patient changed very little from the preoperative blood pressure of 140/90 to 120/80 during and following the dental work. The patient's mother remarked that he had slept possibly 5 minutes in spurts following the previous two visits. His daily chlorpromazine medication was 75–100 mg. per day.

DISCUSSION

These very difficult patients have previously presented severe problems in premedication. The natural hesitancy of the operator to give doses greatly in excess of recommended pediatric amounts has made successful premedication of these children almost impossible. Now the adjunctive use of a sedative-tranquilizing phenothiazine—promethazine—has made it possible to work on three-quarters of these patients in the office. While it was not brought out in the charts and graphs above, there was a definite tranquilizing effect present when promethazine was used with meperidine that was not present when meperidine was used alone. This was noted by many operators and, when it was possible to work on these patients, the work was performed more easily than on those who were premedicated with meperidine alone.

The placebo effect here was also most striking. It is a surprise to the operators that these mentally and emotionally disturbed children experienced approximately the same effects from placebo as did a group of normal, healthy adult patients.[16] Interestingly, one patient experienced dizziness and nausea 25 minutes after receiving a placebo injection. Had this been a drug injection, of course, it would have been considered a side effect.

Concerning side effects, they were not a problem in this study, except in two instances of severe epileptic patients who are on very large doses of sedative agents. Both these patients, when given the combination of meperidine and promthazine, experienced diaphoresis with marked rigidity and clammy skin. Blood pressure dropped only slightly, but the pulse became very rapid. Both patients recovered in a short while without any serious aftereffects. Otherwise, of the fifty patients who received the promethazine-meperidine dombination, seven exprienced other side effects, consisting mostly of nausea without vomiting and dizziness. Ten of the fifty patients who received meperidine also experienced side effects. Four of these patients experienced vomiting; three, excessive sweating; two, oversedation; and the rest experienced nausea and dizziness. Of course, many of these patients experienced more than one side effect at a time. It should be stated here that the side effects in this study were not troublesome except for the two severe epileptics mentioned. It should also be emphasized that, by the use of these doses, dental work could be performed on these patients who so badly needed it and where previously it was not possible. It is our impression that the use of this combination permitted halving the dosage of meperidine, thereby increasing the margin of safety, while achieving better premedication results than the usual dose of meperidine alone.

The hospital patients presented interesting and significant results. Severely retarded patients produce a period of tranquility rather than sedation when observed in an environment other than dental. The dental environment excited the patient and proved the ineffectiveness of the intravenous medication. It was necessary, therefore, to medicate the patient prior to the dental appointment with 3 grains of secobarbital

so as to permit the intravenous promethazine and meperidine to be effective. In all five cases no respiratory depression was observed. It should be understood that the intravenous promethazine and meperidine is a form of intravenous anesthesia and should be given only under hospital conditions and with the assistance of an anesthesiologist. The patient should be observed until reactive before being released.

SUMMARY

By the use of appropriate large doses of promethazine combined with meperidine it was possible to premedicate severely handicapped children so that necessary dental work could be done. The size of the necessary dose varied directly with the degree of retardation. Promethazine replaced half the meperidine, milligram for milligram, and increased effectiveness, while respiratory depression risks were decreased. Side effects were not a problem, except in epileptic patients under medication, where hypotension occurred.

Recommended doses of the combination achieved excellent results in normal office patients. Disturbed patients had a placebo effect remarkably similar to literature reports on normal patients.

Appreciation is given to Dr. L. T. Longtin and his dental staff of the Elwyn Training School and to Dr. L. Levin and his dental staff of the Vineland Training School, Vineland, New Jersey.

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Mechanics in the Production of Mandibular Fractures: A Study with the "Stresscoat" Technique. I. Symphyseal Impacts

DONALD F. HUELKE

Department of Anatomy, University of Michigan Medical School
Ann Arbor, Michigan

A recent study of 319 case histories of mandibular fractures by Hagan and Huelke[1] has shown that certain areas of the jaw are fractured more often than others and that the incidence of certain mandibular fractures is greater when the blow is directed to specific regions of the jaw. Relatively little is known about the response of the mandible to impact, except that, when the magnitude of a blow is sufficient, the bone will break. How does the mandible fracture, and what are the mechanisms involved? These questions have not been answered because of the lack of experimental data. Obtaining these data is an engineering problem involving stresses, strains, impacts, energies, and forces, and thus engineering techniques must be used.

This report, the first of a series of studies on the mechanism of mandibular fractures, presents data on forces and impacts applied to the chin point of the mandible and the resultant deformations of the bone. The results of these tests are correlated with certain clinical findings.

Terminology.—Throughout this report certain terminology generally used in engineering will be employed. Some of these terms need to be defined. The term *force* is defined as a push or pull. The various types of force are illustrated in Figure 1. *Tensile* forces (tension) tend to pull an object apart; *compressive* forces (compression) push the particles forming an object together, while *shearing* forces make one part of an object slide over another part of the same object. When two parallel, oppositely directed equal forces with different lines of action are applied perpendicularly to the long axis of an object, a *torsion* or twisting effect is produced. Here one part of the object is rotated about another part. Forces are measured in mass units (kilograms, pounds, etc.).

Energy is simply defined as the capacity to do work. The magnitude of the energy applied is calculated by multiplying the weight of a falling object by the distance through which it passes.* Energy is then measured in mass-length units (kilogram-

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* Energy of a moving object is usually calculated from the formula $\frac{1}{2}MV^2$, where M is the mass of the object and V the velocity of its motion. Because of the principle of conservation of energy, energy may be determined from the potential-energy formula WH, where W is the weight of the object and H the distance the object has fallen. Because of the difficulty in determining the velocity of the falling sphere, the potential-energy method was used in calculating the energy of impact.

meters, inch-pounds). Force and energy are not synonymous. The term "energy" should be used in dynamic situations involving damaging levels of force, whereas force should be used when the load is statically (slowly) applied.

Stress and *strain* are terms that are often used synonymously and incorrectly. *Stress* is the intermolecular resistance of an object to the deforming action of external forces. It is measured in units of force divided by the area to which the force is applied (grams per square centimeter, pounds per square inch). *Strain* is the change in linear dimensions of an object due to an outside force that has been applied. Unit strain represents the change in length per unit of length in the object (inches per inch, etc.).

EXPERIMENTAL METHODS

Material.—For this study, 27 human mandibles* were used. Included were dentulous and edentulous mandibles and specimens with only the anterior teeth. In addition, each group had individual bones with an obtuse, intermediate, or acute gonial angula-

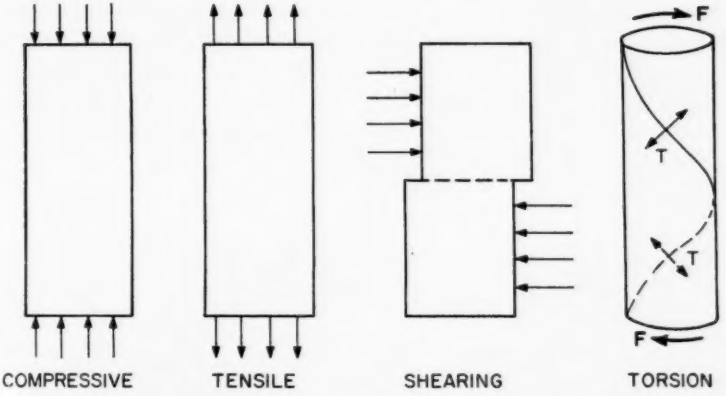

FIG. 1.—The various types of force to which a body may be subjected. Arrows indicate the direction of the force. *T*s on cylinder undergoing torsion indicate the direction of the tension produced by the twisting action. (Modified from Evans, *Ann. N.Y. Acad. Sci.*, **63**:586, 1955, and *Instr. Course Lect., Am. Acad. Orthop. Surg.*, **9**:264, 1952).

tion. Also, certain specimens had very obvious, well-defined, bony markings (ridges, tuberosities, etc.); others were generally quite smooth, with bony markings less heavily textured. No obvious bone pathology was noted. Information on age, sex, and race of the bones was not available.

Theory and technique.—In order to determine visually the areas of highest tensile strain concentration in bone due to a force or impact, the "Stresscoat" technique was employed. "Stresscoat"† is a trade name for a brittle, resinous lacquer developed by deForest and Ellis in 1940.[2, 3] The lacquer is sprayed on the bone and allowed to dry 15–20 hours before testing. Steel calibration bars are coated along with the test specimens. Just prior to the bone tests, individual bars are tested as a cantilevel beam and

* From the osteological collection of the Department of Anatomy, University of Michigan Medical School.

† Magnaflux Corporation, Chicago, Ill.

placed in a scale that gives a direct reading of the lacquer sensitivity in inches per inch (Fig. 2). It was assumed that the sensitivity of the lacquer was the same on both the bars and the bones, for both were dried under the same temperature and humidity conditions.[4] The sensitivity of the lacquer used in the tests varied from 0.005 to 0.013 inch/inch [500–1300 micro inches/inch (MII)]; the majority of the bones were tested with a lacquer sensitivity of 600–800 MII.

Stresscoat cracks in response to the tensile strain in the underlying material on which it has been sprayed, with the cracks lying perpendicular to the direction of the strain. The cracks always appear first in the area of highest tensile strain. When material such as bone breaks more readily in tension than in compression,[5] failure (fracture) will occur at a region of highest tensile strain. It has been found[6] that the Stresscoat deformation patterns on dry bones (skulls) are similar to those produced in living animals under similar test conditions. The major differences are that deformation patterns are more extensive in the living animals and the patterns tend to be interrupted by suture lines in the dry skulls.

Immediately before testing, the bone is coated with a penetrant fluid. When the force or energy is applied, the penetrant liquid flows into the lacquer cracks; after testing, the surface of the bone is wiped dry, the penetrant liquid remaining only in the cracks. The bones are then sprayed with an electrostatic powder,* which, attracted by the penetrant fluid, collects in the cracks, making them plainly visible. The patterns are then inked and the bones photographed.

*Statiflux, Magnaflux Corporation.

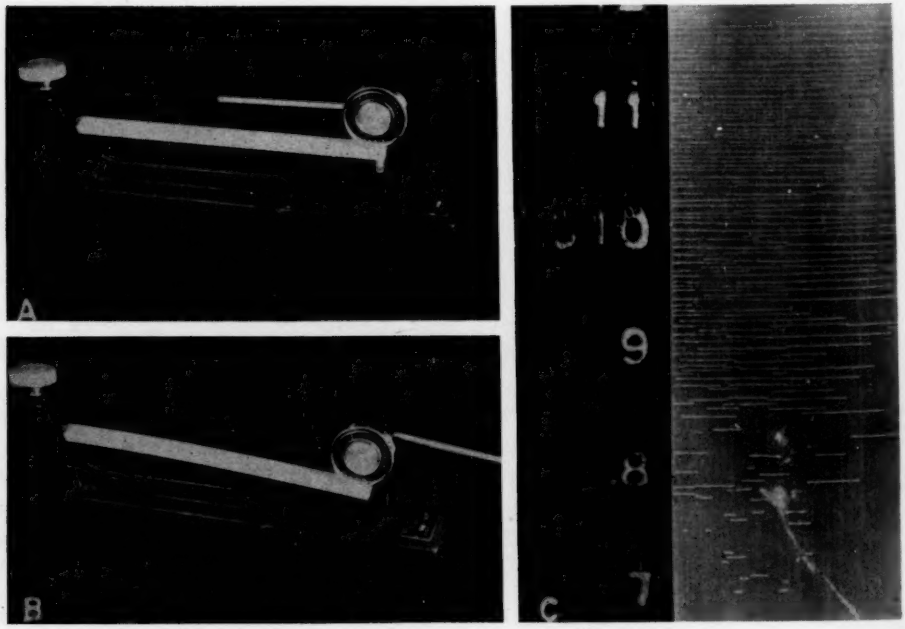

FIG. 2.—Method of determining lacquer sensitivity. *A*, Stresscoated metal bar ready to be tested. *B*, bar bent by deflection of handle. *C*, close-up of Statifluxed bar in scale; lowest lacquer crack at 7, indicating lacquer sensitivity at 0.0007 inch/inch.

In the static loading tests a force of 25–70 pounds was applied to the mid-line of the chin at the inferior border of the bone.The time of loading was 1 minute. All bones were oriented so that a line through the chin point and condyle was perpendicular to the horizontal base plate. Static loadings were carried out in a Riehle testing machine with an accuracy of 1.0 per cent (Fig. 3). The condyles were placed in cupped steel blocks and the chin point covered by a small strip of leather to prevent the bone from slipping away from the force applicator, which had a diameter of 0.68 inch. A total of 103 static loadings was made on the 27 mandibles.

In the dynamic testings the bones were oriented as above, but the method of condylar fixation varied. In one group of tests the condyles were imbedded in cupped base plates filled with clay. These base plates were mounted in tracks, so that, when necessary, the base plates could be moved according to the intercondylar distance of the individual test specimen. The tracks were welded to a $\frac{1}{8}$-inch steel plate weighing 94 pounds (Fig. 4). Because of the mass of the plate, the energy absorbed by it was negligible. In this manner, 191 tests were completed.

In another series of tests the condyles were imbedded in a solid bar of Woods metal —a low-temperature alloy—and placed in a 41-pound bench vise, which was also mounted to the steel plate (Fig. 4). Using this type of condylar fixation, 146 tests were performed.

In the third set of tests, an area of the Woods metal bar under the left condyle was

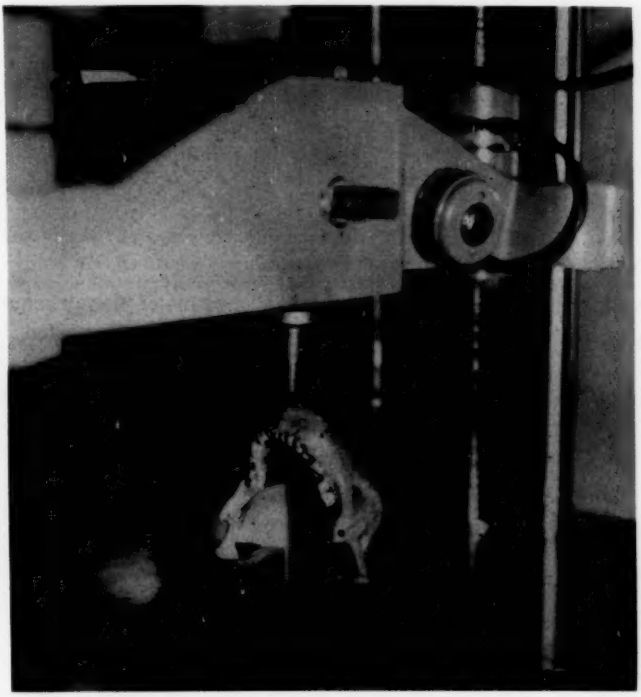

FIG. 3.—Close-up of Riehle testing machine, mandible under static load

removed and filled with clay, and the Woods metal bar was fixed in the bench vise (235 tests). Thus in these dynamic tests the condyles were free to move (imbedded in clay), firmly fixed (metal), or one condyle fixed, with the other free to move. The load was dynamically applied to the mandible by dropping a steel ball, 1 inch in diameter and weighing 0.146 pound, through a metal tube 0.69 foot in length. The energy applied was 0.101 foot-pound. The friction between the ball and tube was negligible. Preliminary testings indicated that this amount of energy, although small, was sufficient to crack the lacquer in the areas of high tensile strain. Experimental studies, using the Stresscoat technique, wire-resistant strain gauges, and loading the bone to failure, have shown that linear fractures of the skull, pelvis, and femur arise from failure of the bone due to tensile stresses and strain within it at the initial areas of high-

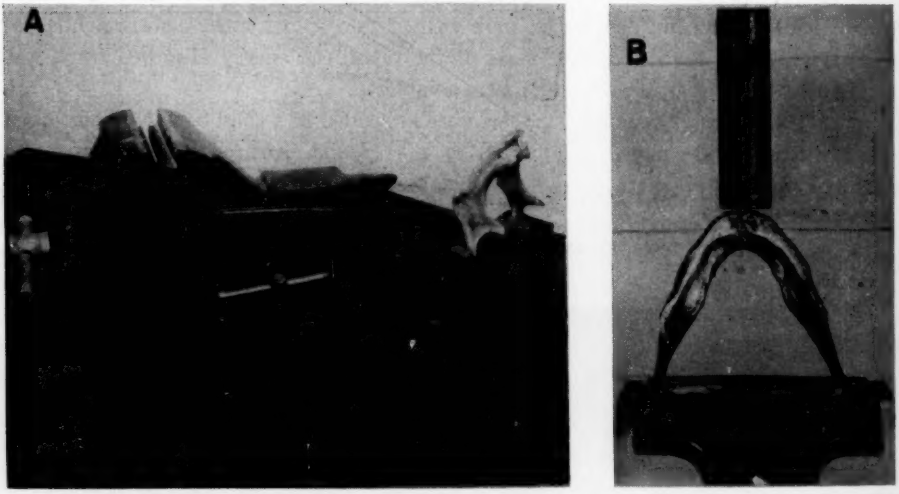

FIG. 4.—*A*, apparatus used in dynamic loadings. Note mandible in clay-filled base plates. *B*, close-up of mandible mounted in bench vise with tube, through which the weight was dropped, over the point of load application.

est tensile strain.[7–13] Therefore, only the minimal amount of energy required to crack the lacquer was used. When a greater magnitude of energy was applied, but not sufficient to break the specimen, extensive deformation patterns appeared, and it was not possible to determine which regions were the initial high tensile strain areas.

RESULTS

Although each region showing a deformation pattern will be described separately, it must be remembered that these strain patterns usually appeared on each individual bone. That is to say, the bone reacts to impacts as a unit, not as isolated parts. The areas involved are (1) subcondylar region, (2) lingual side of chin, (3) buccal or labial alveolar walls, (4) mental foramen, (5) miscellaneous areas.

Subcondylar deformation patterns occurred more frequently than any other. Most often the lacquer cracks were found on the lateral side of the condylar neck (Fig. 5), indicating that the condyles were bent medially, thereby producing high tensile strain

on the lateral side. With an increase in load, the subcondylar patterns became more extensive and spread down the ramus by further outbending of this region (Figs. 5 and 9).

When the medial end of a condyle was in contact with the side of the base plates, only an outward bending of the condyle was possible. In these cases a deformation pattern was found on the medial side of the neck of the mandible (Fig. 6).

With both condyles imbedded in metal, a medial or lateral bending of the condyle was not always noted. In approximately 40 per cent of the tests, there were deformation patterns located on both sides of the neck, with the lacquer cracks on the lateral side parallel in direction to those on the medial side (Fig. 6). This indicates that tension was produced on both sides of the subcondylar area. With the condyles fixed in metal, they are unable to move. The movement necessary to produce these subcondylar deformation patterns must then be in the neck itself. In these cases there is an oscillation in the subcondylar area, which first bends in one direction and then in the op-

FIG. 5.—*A*, typical lateral subcondylar deformation pattern with other deformation patterns about the buccal alveolar walls, mental foramen, and lateral chin regions. *B*, extensive subcondylar deformation pattern.

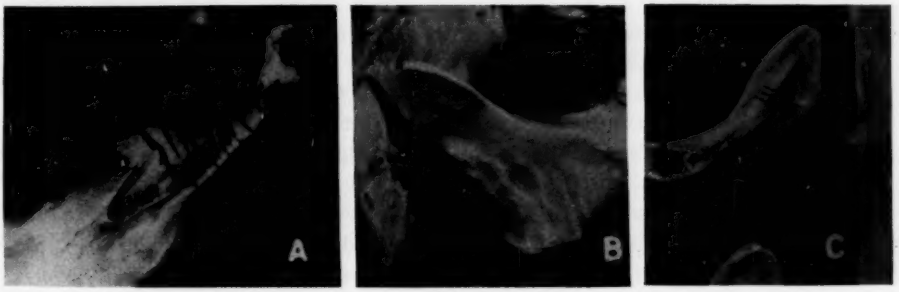

FIG. 6.—*A*, medial subcondylar pattern, indicating an outward bending of the condyle. *B*, lateral subcondylar and coronoid process patterns. *C*, medial subcondylar and coronoid process patterns from same bone as that shown in center picture. Medial and lateral deformation patterns on the same side indicate a vibration of the neck of the mandible and coronoid process.

posite direction, beyond the resting position of the bone. Also with this type of condylar fixation, the occurrence of deformation patterns on only the medial side of the condylar neck was quite high, in about one-third of the tests.

In addition to the subcondylar patterns described above, bones, with condyles imbedded in metal also had posterior subcondylar patterns (6 per cent), indicating a stretching of this area. This high tensile strain along the posterior border of the neck of the mandible is due to a forward bending of the condyle (Figs. 7 and 8). Rarely (3 per cent) was there a twisting of the condyle noted. Here the lacquer cracks of the lateral aspect of the neck are perpendicular in direction to those found on the medial side.

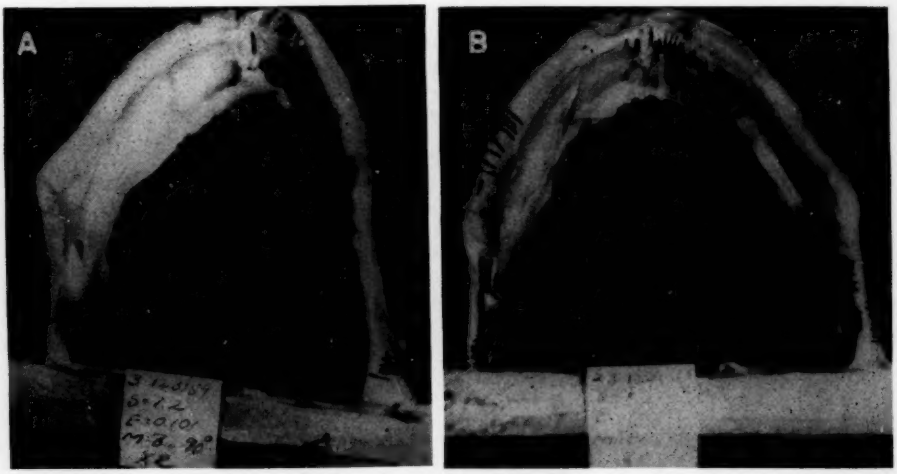

FIG. 7.—*A*, minimal posterior subcondylar and lingual chin deformation patterns, condyles fixed in metal. *B*, more extensive posterior subcondylar patterns, with inferior angle pattern on the right side and extensive lingual chin pattern on the left. In this case the right condyle was imbedded in metal and the left condyle in clay.

When one condyle was imbedded in metal and the opposite condyle in clay, the subcondylar deformation patterns were not always the same on the two sides. Usually both condyles had deformation patterns on their lateral sides, indicating a medial or inward bending. However, in about 20 per cent of these tests the subcondylar area of the metal-imbedded condyle vibrated.

Vertically oriented deformation patterns were found on the lingual aspect of the chin (Figs. 7 and 8). From the energy applied to the labial side of the symphysis, tensile strain was produced by an inbending (flattening) of the chin and a concomitant stretching of the lingual cortical plate. The cracks were not always grouped on the mid-line, but rather they were located adjacent to the mental spine and about it. The cracks rarely extended through the mental spine, although they were found on the apex of the spine. Occasionally, part of the deformation pattern continued from above the spine and around it to reach again close to the mid-line in the lower half of the chin. This indicates that the greatest amount of tensile strain in the chin area is not produced directly on the mid-line but is paramedian in location and that the symphysis is

somewhat resistant to stretching, owing, in part, to the strengthening effect of the mental trigone labially and the mental spine lingually. With one condyle fixed in metal and the other free to move in clay, the lingual chin pattern was more extensive on one side—the side on which the condyle was free to move—indicating that a greater amount of tensile strain was produced here on this half of the bone and that the other half of the mandible was more rigid (Fig. 7).

The buccal alveolar walls tend to concentrate stresses. This is due to the sharp edges of the alveoli, the thinness of their walls, and the fact that the curve of the buccal alveolar wall is superimposed on the outer curve of the mandibular arch, which is, in general, the tensile side of the bone. Tensile strain cracks were found extending from

FIG. 8.—Typical posterior subcondylar and lingual chin patterns with minimal inferior angle deformations on the left side and an extensive angle pattern on the right side.

the edge of the buccal side of alveoli, indicating a mesial-distal compression of the alveoli, with stretching of the outer walls (Fig. 5).

High tensile strain was developed about the mental foramina, especially in static tests and dynamic tests in which the condyles were imbedded in clay. Here the deformation pattern appeared as cracks distributed radially about the foramen produced by a stretching of the bone in a circumferential direction around its margin (Fig. 9). Less extensive mental foramen patterns, where only a few cracks appeared, indicate that the stress concentration occurred in only one or two places on the edge of the foramen.

Less frequently—in 20–35 per cent of the tests—patterns were noted about the lateral chin region, oblique line and buccal shelf, alveolar ridge, lingula, and lingual tuberosity. Deformation patterns located between the chin point and the mental foramen—the lateral chin region—were usually found with patterns about the mental foramen. When the chin was flattened, high tensile strain was produced in this area near the mental foramen.

The oblique line, buccal shelf, and alveolar ridge showed deformation patterns produced by bending and/or twisting actions. In these areas, patterns perpendicular to the long axis of the bone indicate that high tensile strain was produced by an outward bending of the oblique line, buccal shelf, and lateral part of the alveolar ridge (Figs. 9 and 10). Obliquely oriented cracks in these areas (Fig. 10) indicate a twisting of the body on the ramus, with the alveoli or alveolar ridge turning downward and lingually. In addition, a slight amount of twisting was occasionally found in the subcondylar region, as noted previously.

Lacquer cracks about the lingula indicate a concentration of high tensile strain, although this area is on the compressive side of the mandibular arch. Likewise, the lingual tuberosity on the medial side of the third molar tooth had a deformation pattern,

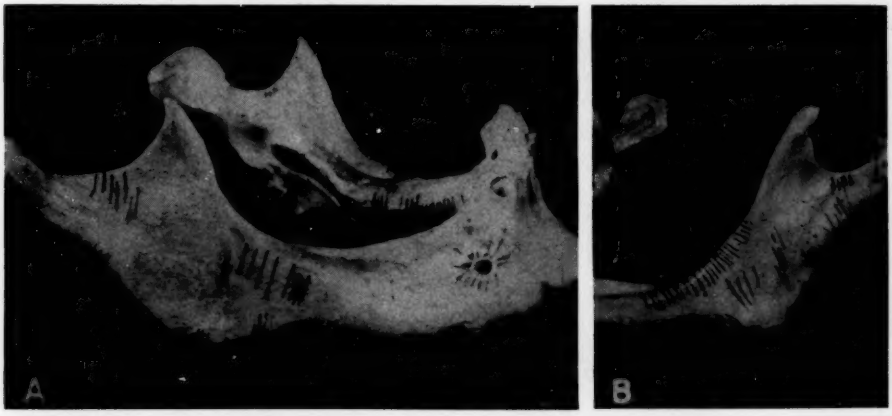

FIG. 9.—*A*, subcondylar, oblique line, and mental foramen deformation patterns. *B*, similar patterns but more extensive than those in *A*.

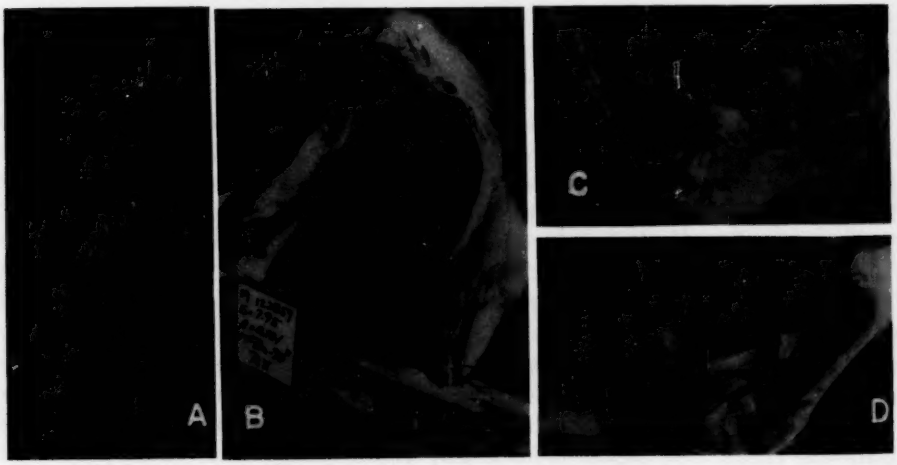

FIG. 10.—*A*, oblique buccal shelf and alveolar ridge deformation patterns. *B*, alveolar ridge patterns perpendicular to the long axis of the bone. Patterns about the lingula (*C*) and lingual tuberosity (*D*).

indicating that tensile strain arose from a bulging of this area lingually (Fig. 10). Deformation patterns at the base of the coronoid process were noted in testings with at least one condyle imbedded in metal. The patterns were found on the lateral or medial side or on both sides of the base of the coronoid process (Fig. 6). Here the process is bent either inward or outward, or vibration of the process takes place. The majority of the coronoid process deformation patterns were found on 10 bones, most of which were edentulous specimens. This vibratory phenomenon was noted more often when both condyles of the test specimen were imbedded in metal.

When both condyles were imbedded in metal, symphyseal impacts produced high tensile strain in the region of the antegonial notch (Figs. 7 and 8). These patterns were more frequent in edentulous specimens. In these cases the tensile strain is produced by a stretching of the bone parallel to and along the base of the mandible in the region of the antegonial notch, patterns which at times extended forward along the base of the mandible in front of the antegonial notch.

DISCUSSION

Up to this time, very few mandibles have been tested to determine their response to forces and impacts. In 1935, Küntschner,[14] using Colophonium (an early type of strain-sensitive lacquer), found deformation patterns about the mental foramen and adjacent buccal alveolar walls. These patterns were vertically oriented and were due to a "bending load." The method of application of the load was not stated; only one bone was tested. Evans,[15] using Stresscoat, statically loaded two mandibles; one specimen had unilateral subcondylar and inferior angle tensile strain deformation patterns produced by a static loading of 59 pounds. This force was applied to the chin point in a line parallel to the long axis of the body. In another specimen, a force of 175 pounds was applied to a brass rod laid across the inferior body of the mandible near the angle; in this test there was an extensive unilateral medial subcondylar pattern with a pattern on the lingula. DuBrul and Sicher[16] found high tensile strain patterns between the mental foramina, produced by squeezing the condyles of one mandible together with finger pressure. Ueno, Oka, Miyagawa, and Kobayashi,[17] using Stresscoat and strain gauges, tested mandibles and acrylic models by applying forces and impacts to the symphysis and angle regions. The number of tests is not indicated in their report. Static loadings applied to the symphysis of acrylic models revealed high tensile deformation on the lingual aspect of the chin and the lateral side of the subcondylar, ramal, and angle regions. These patterns are similar to those found in the present study. Sharry, Askew, and Hoyer,[18] using Stresscoat on dry skulls, found deformation patterns on the mandible, maxilla, and adjacent facial bones following simulated biting forces on complete dentures.

The energy of a blow to the symphysis causes the condyles to be seated in the joint cavities. The question now is, "Are the condyles movable?" There are several possibilities: (1) they may be firmly fixed; (2) although forced into the joint cavities, they may be relatively free to move as the condyle slides on the disk; (3) the condyle-disk unit may slide in the mandibular fossa. Experimentally, an attempt has been made to duplicate these possibilities by firmly fixing the condyles in metal or allowing the condyles to move by putting them in a soft medium (clay).

The response of the mandibles to symphyseal impacts is dependent, in part, on the

method of condylar fixation. If the condyles are relatively free to move, i.e., imbedded in clay, the mandible behaves as an engineer's pin-jointed arch (Fig. 11). Application of the force at the center of the arch subjects the entire outer surface of the arch, except at the point of impact, to tensile stresses and strains. However, as found experimentally, the strain is concentrated in the subcondylar and mental foramen areas and along the buccal alveolar walls, the greatest amount of deformation occurring in the subcondylar areas. Tension also is developed on the inner aspect of the curve of the arch (the lingual chin area) as the result of the flattening of this region by the energy applied to the opposite side.

When the ends (condyles) of the arch are imbedded in metal they are unable to

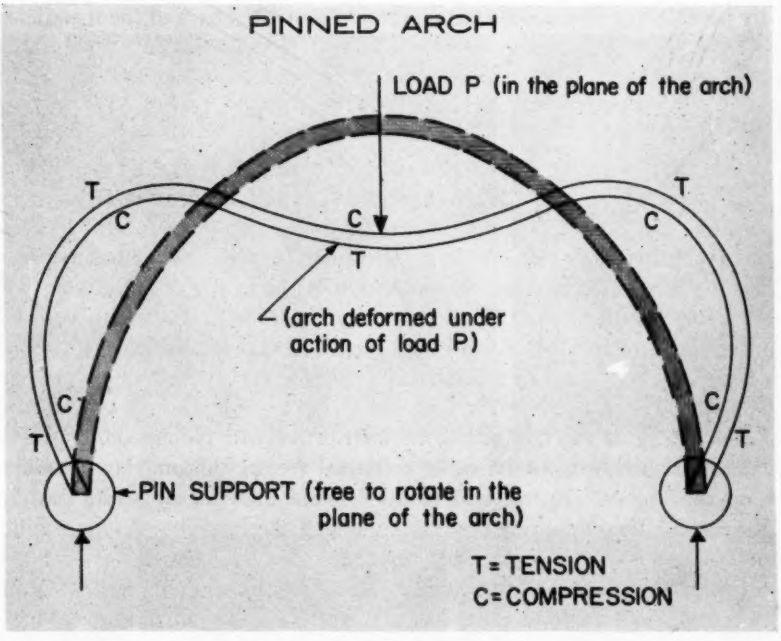

FIG. 11.—The effect of load on an arch the ends of which are free to move

move, and the response of the bone is somewhat different. Although flattening of the chin area and outward bending in the mental foramen areas still occur, there is an inward bending of the limbs of the arch near its fixed ends (Fig. 12). In the dynamic tests, with the condyles imbedded in metal, this was evidenced by the medial subcondylar deformation patterns. Even more frequently observed was a vibratory phenomenon, evidenced by deformation patterns on both the medial and the lateral sides of the same subcondylar area, the result of an undulating movement of the subcondylar region itself.

When the right condyle is imbedded in metal and the left in clay, the response is in some respects similar to a combination of results of the other dynamic testings described above. In general, the entire arch shifts to the left—the side of least resistance. This occurs by a bending in three areas: the lateral aspect of the right and left sub-

condylar regions—as evidenced by deformation patterns located here—and by a flattening of the lingual aspect of the chin, especially on the left side of the mid-line. This latter pattern indicates that the entire left half of the arch is moving laterally, with the tension, produced by the movement, located in the left lingual chin region. When the bone rebounds from the impact, frequently the mandibular arch goes beyond its rest position, so that there are high tensile stresses developed on the medial aspect of the right subcondylar region.

Marked surface irregularities will tend to concentrate stresses. These irregularities take the form of projections (lingulae), bulges (lingual tuberosity, alveolar walls),

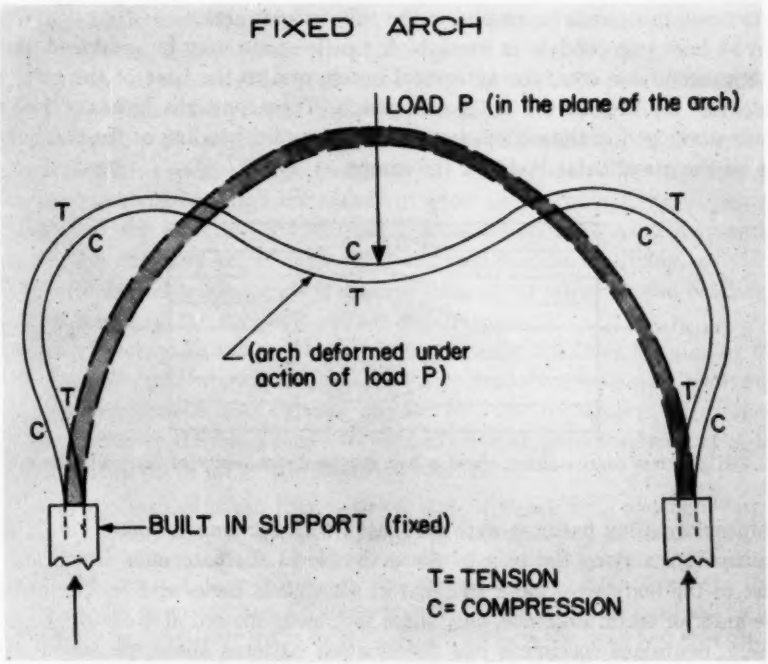

FIG. 12.—The effect of load on an arch with the ends unable to move

and foramina (mental foramen). They frequently have sharp edges, a short radius of curvature, discontinuities, and/or are usually the points farthest from the neutral axis of the bone. The lateral alveolar walls frequently had deformation patterns. Because of the sharp edges of the alveoli, the short radius of curvature of their walls, and their superimposed position on the mandibular arch, tensile stresses build upon only the buccal alveolar walls, the lingual side of the alveoli being on the compressive side of the mandibular arch. The deformation patterns indicate an outward bulging of the lateral alveolar walls or high tensile strain values produced in the lateral walls by a mesial-distal compression of the alveoli. Stresses develop about the circumference of the mental foramen because of the discontinuity of the material and concentration of stress in a localized region (Fig. 13). Reduction in the cross-sectional area through the foramen also gives a greater stress per unit area value, thereby increasing the

amount of strain in this area. The lingual tuberosity and the trihedral eminence, located below the anterior extremity of the oblique line, tend to concentrate stresses. This is due to the fact that the stress is highest in regions that are farthest from the neutral axis.

When the load is applied to the symphysis and to the condyles (by the reactive load), the energy is transmitted into both limbs of the arch. The condyles are not directly beneath the point of load application; instead, the symphysis is medial to them. This type of eccentric loading at times produced a torsion somewhere along the limb of the arch. This appeared as the angulated, oblique course of the lacquer cracks along the oblique line and buccal shelf and in some of the alveolar ridge deformation patterns. At times this torsion appeared in the subcondylar area.

When at least one condyle is fixed, high tensile strain may be produced along the posterior subcondylar area, the antegonial notch, and to the base of the body of the mandible just anterior to the antegonial notch. These patterns indicate that tensile strain was developed in these areas because of a forward bending of the condyle and a bending of the mandibular body on the ramus.

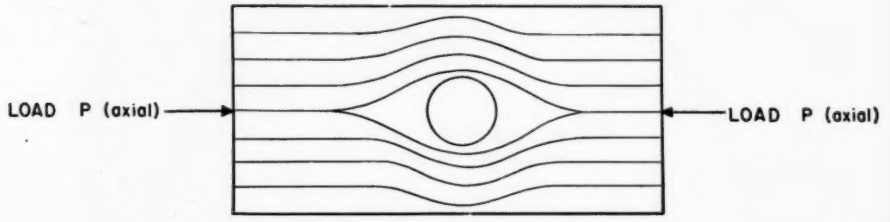

FIG. 13.—High-stress concentration about a hole due to discontinuity of material with same load, *P*, acting.

Certain deformation patterns were correlated with the state of dentition. High tensile strain patterns along the base of the mandible in the antegonial notch and adjacent base of the body were more frequent in edentulous bones and in specimens with only the anterior teeth. Likewise, only these specimens showed alveolar ridge patterns. Conversely, dentulous specimens had deformation patterns about the lateral alveolar walls.

In their study of 319 cases of mandibular fractures, Hagan and Huelke[1] showed that more than half (56.2 per cent) of the fractures resulted from impacts to the chin (between the mental foramina), with 22.8 per cent of all fractures due specifically to chin point (symphyseal) blows. In their study, the subcondylar area was the most frequently fractured region of the mandible (36.3 per cent). Furthermore, they have shown that all areas of the mandible can be fractured by impacts to the symphysis. In another study, Huelke, Burdi, and Eyman[19] have shown that the association between the site of trauma and the location of fractures, especially in individuals with complete dentition and those with only a few individual teeth missing, is highly significant and due only to chance in one out of 1,000 randomly selected cases. Experimentally, the results of the Stresscoat tests have indicated that high tensile stresses and strains can be produced in all areas of the mandible, depending on the method of condylar fixation. The analysis of clinical data indicates that there is a definite relation

between the location of the fracture and the site of trauma. For example, most subcondylar fractures (71.4 per cent) are due to blows to the chin region, with 38.8 per cent due to chin-point impacts. In addition, impacts to the chin point cause more fractures per impact than those to any other region, the ratio between the two in the case of chin-point blows is 1:2.2.

Fractures of the coronoid process are rare (2.1 per cent) and are almost always associated with other mandibular fractures. Direct trauma to the coronoid process is secondary to fracture of the zygomatic arch or the maxilla of the same side. In these cases the process is forced against the bone of the infratemporal fossa or if the teeth are in occlusion, thereby holding the process stationary, by the force of the medially displaced zygomatic arch. Impacts to the ramus or body of the mandible of the same or opposite side can cause coronoid process fractures by producing fractures in other areas, so that the segment of the arch containing the coronoid process in question is displaced. This displacement forces the process against the bone of the infratemporal fossa or zygomatic arch and subjects it to a high bending moment. In this experiment, symphyseal impacts produced high tensile strain at the base of the coronoid process when at least one condyle was imbedded in metal in approximately 40 per cent of tests. In general, the patterns indicate that a vibration occurred more frequently than a bending either medially or laterally. It is assumed that in the living individual the temporalis muscle and tendon have a marked dampening effect on the bending or vibratory movements of the coronoid process due to impacts.

The factors involved in the production of mandibular fractures are similar to those producing cranial vault injuries. Blows or impacts producing fractures involve energy, velocity, and deceleration. "All injuries are the result of the absorption of energy."[12] This occurs whenever the head comes in contact with an object moving at a different velocity. This may occur under the following conditions:

> (1) The resting head, although free to move, may be struck by a moving object and be accelerated, (2) the moving head may strike a resting object and be decelerated, (3) the moving head strikes or is struck by a moving object. In this instance the head may be accelerated or decelerated depending on whether the head or the object is moving the faster and also on the relative masses and directions of each. The injury to the head will be identical in each of the three conditions if the location of the blow on the head and also the relative velocity between the head and the object is the same.[20]

SUMMARY

Forces and impacts to the symphysis of the mandible cause deformations which are specific in their location. Most frequently these impacts cause a deformation of the neck of the mandible, the type of deformation depending on the method of condylar fixation. This deformation may be a medial or lateral bending of the condyle or a vibration of the subcondylar region itself. In addition, the lingual aspect of the chin tends to flatten because of the application of the force to the opposite side of the bone. Tensile stresses and strains tend to concentrate about the mental foramen, buccal alveolar walls, lingula and lingual tuberosity, oblique line, buccal shelf, and inferior margin of the mandible. However, following chin impacts, these areas are not so frequently involved either clinically or experimentally. Additionally, the coronoid process tends to vibrate because of chin impacts when the condyles are firmly fixed. Clinical data have shown that any area of the mandible may be fractured by impacts to

the chin region, with the subcondylar region being most frequently involved. The experimental results of the present study also indicate that all areas of the mandible may develop high tensile stresses and strains due to impacts to the chin and that the subcondylar region is most frequently involved.

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The Accuracy of Large Castings

R. H. ROYDHOUSE and E. W. SKINNER

Eastman Dental Dispensary, Rochester, New York, and Northwestern University Dental School, Chicago, Illinois

The accuracy of cast partial dentures was investigated by measuring various dimensions of the investment cast and the metal casting. While other investigators have been concerned with inlay casting and the changes in dimension of gypsum products and cast alloys, the influences upon the accuracy of large castings has not been well defined. Eighty investment models and castings made under different known conditions were measured, and the information was summarized by statistical techniques. On the basis of the data, two hypotheses were formed and tested. One change in technique was made to improve the accuracy, and hence fit, of the castings. The shape and design of the master model or die was associated more with measuring needs than with clinical verisimilitude.

The report is divided into two parts—the investment cast and the casting process.

PART I. THE INVESTMENT CAST

MATERIALS AND METHODS

Two master models, made from Invar, were used and represented lateral cross-sections through mandibular or maxillary casts similar to those sent to the dental laboratory by the dentist for fabrication of a metal framework for a partial denture. Model A was cut on a lathe, and the "faces" of the ridges were segments of a sphere, with the center at the midpoint of the base. Model B had flat faces on the ridges (Fig. 1). The dimensions are given in Figure 2.

These master models were duplicated in a conventional laboratory flask; the model was attached to the base with plasticine or similar material, the top half of the flask placed in position, and hot reversible hydrocolloid* poured in. The assembly was then cooled by placing the base in running water (60° C.). As soon as the hydrocolloid had cooled and jelled, the base and master model were removed from the assembly, and the investment† material was vibrated into the mold space.

This study was partially supported by a grant from the Office of the Surgeon General, Department of the Army. The technical work was supervised by J. Osborne, R. Earnshaw, B. Hedegaard, and the authors at the Dental School, Northwestern University.

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* Deelastic duplicating material was used for all procedures connected with gold castings. For the casts upon which cobalt-chromium castings were made, DP Ticonium Special Duplicating material was used. For all materials the manufacturers' instructions were followed, unless otherwise stated.

† Five investments were used in the gold-casting procedures; the code is given in the publication by Osborne and Skinner (ref. 1). Ticonium C51 Investic was used for cobalt-chromium procedures. A water-to-powder ratio of 0.30 was used throughout, unless otherwise stated.

After the investment had set, the investment cast was removed and allowed to dry for at least 24 hours. The specimen was then leveled on a tripod and measured in two dimensions. In the vertical dimension—that is, axial or parallel to the long axis of teeth —a dial gauge (0.01 mm. accuracy) with a pointed shaft was used to establish the relations between six places on each cast. At each place six readings were taken and averaged; by subtraction, the vertical distances between three places on each side were ascertained. In the horizontal (lateral or occlusal dimension) a comparator microscope ($\pm$0.001 mm.) was used to measure the separation of indentations appearing on

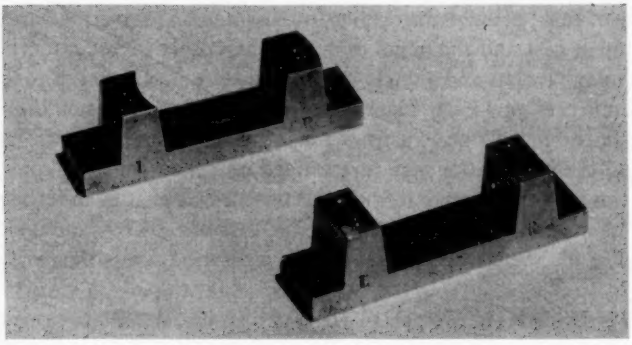

FIG. 1.—The two Invar models. Model A has curved sides to the alveolar ridge. Model A is plane or flat in all surfaces.

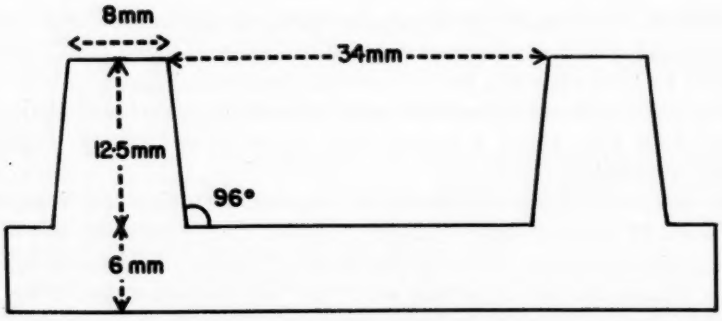

FIG. 2.—Dimensions of models

the "occlusal" surface of the ridges; six readings obtained by traversing left to right and six, right to left, were averaged.

For ease of statistical manipulation, further averaging was undertaken. The means for various distances on the casts were subtracted from the identical dimensions on the relevant master model, and the difference or error was expressed as a percentage of the model dimension. Further, the assumption that symmetry about the center of the casts (and castings) existed was tested by means of a regression equation where right and left sides were related; the coefficient of correlation was 0.93–0.95. Therefore, the values for either side were averaged. The values used throughout as original or raw data for the calculations and the tables are therefore means of twelve measurements ex-

pressed as a percentage error; I, II, III denote vertical measurements and IV is the horizontal measurement (Fig. 3).

Using I, II, III, and IV, an analysis of variance was made; three or more casts were used in each category. All values were accepted, and no criteria for selection were used. The statistical methods were used to summarize the data, to separate various groups of measurements, and to verify hypotheses.

RESULTS

Different investments.—Measurements of casts made of six different investments were analyzed. No significant difference existed between the means of the horizontal dimension for model A with different investments. Table 1 shows the average for measurements I, II, and III combined for each investment (four casts each). The rank from least to greatest in vertical increase of investment cast was T, E, B, A, F, D. These results are compared in Table 2 with those reported by Osborne and Skinner;[1] in that investigation the setting expansion of these investments (same batches) was determined by a dial gauge upon a cover slip on the top of investment in a small asbestos-lined casting ring; their order, least to greatest, was D, A, E, F, B. Coincidence in

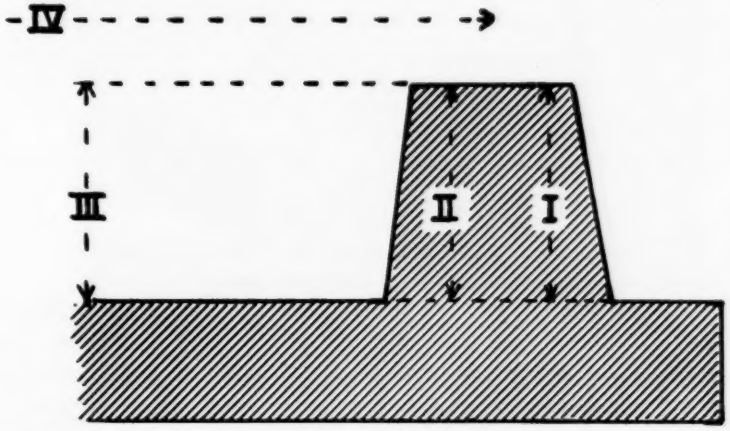

FIG. 3.—The averages for the distances I, II, III, and IV were used as basis for statistical analysis.

TABLE 1*

INCREASE IN VERTICAL DIRECTION OF INVESTMENT CASTS

(Average Values for Six Investments)

	INVESTMENTS					
	A	B	D	E	F	T
Per cent increase.....	0.95	0.85	1.30	0.69	1.17	0.64

* Each mean represents an average of 48 separate measurements, and the 5 per cent confidence limit for the difference between two means is 0.26.

values for expansion is not expected, but the change in position of D and E is noted.

Method of duplication.—The duplicating medium was altered in one experiment, and a rubber-based impression material was used instead of reversible hydrocolloid; the results when investments B and E were used are shown in Table 3.

Geometry of models.—The "alveolar" ridge of model A had curved sides, whereas model B was flat in this region. The effects of model geometry are shown in Table 4, where investment T alone was used.

Position of model within flask.—The previous results led to an experiment in which the position of the master model in the flask and the thickness of the base were varied. The following variants were tried.

a) Standard position (SP); the model in position resembled a cross-section through

TABLE 2

RANKING OF INVESTMENT BY AMOUNT OF EXPANSION IN VERTICAL DIRECTION

	T	E	B	A	F	D
Investment casts.....	0.64	0.69	0.85	0.95	1.17	1.30

	D	A	E	F	B
Laboratory test.....	0.70	0.94	1.10	1.12	1.20

TABLE 3

DUPLICATING MEDIA: MEANS OF PERCENTAGE CHANGES (Changes in Vertical Dimension)

Material	Investments		Material	Investments	
	B	E		B	E
Hydrocolloid.....	0.77	1.30	Rubber-based impression.	0.75	0.91

TABLE 4*

CHANGES USING TWO DIFFERENT MASTER MODELS (Means of Percentage Changes)

Models	Measurements			
	I	II	III	IV
A.....	0.67	0.56	0.69	0.00
B.....	1.26	0.88	1.06	0.32

* The 5 per cent confidence limit for the difference between two means is 0.24.

the posterior region of a partial denture cast; the alveolar sections, therefore, represented molar segments (Fig. 4).

b) Standard position, base doubled in thickness (SP2); the position was as above, but the base of the model was increased to about ½ inch in thickness by the addition of a brass plate.

c) Standard position, rotated (SPRT); the master model was turned in the mold so that it lay in the anteroposterior direction and thus represented an anterior segment of a partial denture cast. The subnotation /A indicates a colloid-to-water ratio 1:3 by volume (used throughout) and /B represents a ratio of 1:2. The latter ratio was a departure from the manufacturer's instructions, to ascertain the effects of a "stiffer" jell.

d) Position CC; a special duplicating flask in which ½ inch of jell surrounded the specimen on three sides was used.

e) Standard position, rotated over (SPO); the model was rotated forward about the

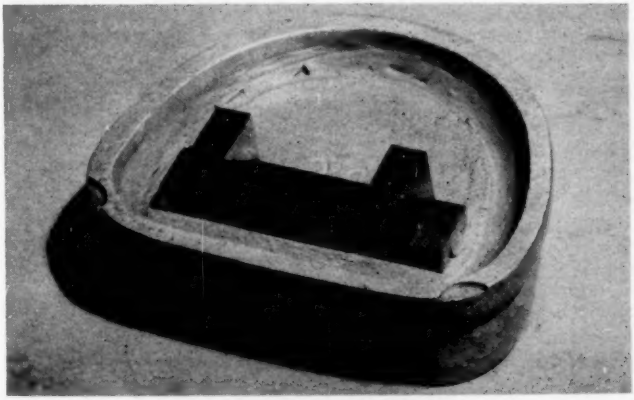

FIG. 4.—The Invar model is in the *standard position* in the conventional flask

lateral axis so that it lay on its side in the flask. The direction of least restraint upon the expansion of the investment was at right angles to the direction of a "tooth" (Fig. 5).

f) Standard position, rotated over, base twice the thickness (SPO2); in position, the model was as above in *e*, but the base was increased in thickness.

In Table 5 the positions of the master model with corresponding increases in I, II, III, the vertical dimensions, and IV the horizontal dimension of investment casts, are given.

DISCUSSION

In Table 1 the change in vertical dimension is shown; changes in horizontal dimension were small, and the investments did not differ significantly. Measurement of setting expansion in a casting ring did not produce the same order of values as the investment casts (Table 2). Hence the geometry of the system (shape and size of parts) is an important, if not overriding, influence on the increase in any dimension during the setting expansion of investment.

In another paper[2] Earnshaw shows that a rod of investment E (not the same batch) surrounded by hydrocolloid gave a setting expansion of 0.62 per cent measured parallel to the rod. The methods of measurement and the geometry of the systems can cause confusion. Investments T and E are reputedly different only in oxalate content. Table 6 illustrates the point to be made, using values for setting expansion from Earnshaw[2] and this work.

In establishing a test for setting expansion where the results must be reproducible, the tendency has been to oversimplify, and the results of these laboratory tests may not be a guide to the use of the investments in service conditions.

The importance of geometry is further emphasized in Table 4; in model A contrasted with model B, the greater surface area or the curved nature and some interlocking have repressed vertical expansion.

The sensitivity to hygroscopic setting expansion of any investment may allow the surrounding media to influence the amount of expansion. In Table 3 a change in surrounding medium did not affect investment B but in non-aqueous media the expansion

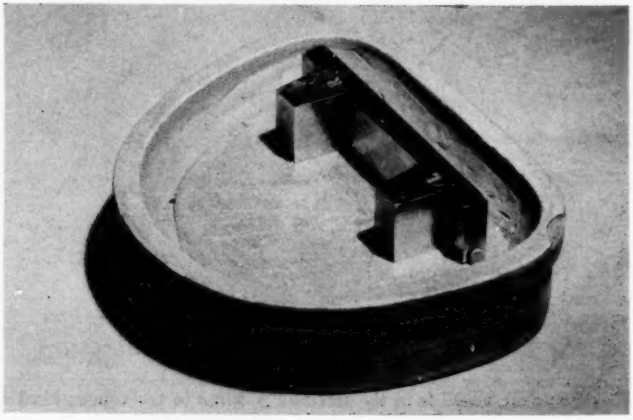

FIG. 5.—The Invar model has been rotated over on its side (SPO)

TABLE 5*

POSITION OF MASTER MODEL

(Means of Percentage Errors)

POSITION	MEASUREMENTS			
	I	II	III	IV
SP.....	1.52	0.97	1.18	0.16
SP2.....	1.26	0.81	0.92	0.33
SPRT/A...	1.32	0.83	0.99	0.05
SPRT/B...	1.37	0.80	1.02	0.13
CC.....	1.30	0.95	0.94	0.11
SPO.....	0.75	0.67	0.71	0.55
SPO2.....	0.52	0.54	0.52	0.66

* The 5 per cent confidence limit for the difference between two means is 0.26.

of E decreased. Thus investment E was, in this case, noticeably sensitive to the presence of water in the surrounding media, whereas B was not.

The difference between horizontal and vertical measurements led to the experiment in which the investment with the least amount of expansion (T) was used and the position of the master model was varied (Table 5). When the dimension under scrutiny was confined at either end by reversible hydrocolloid, the setting expansion was markedly less than when freedom to expand at one end existed. Measurement IV of category SP can be contrasted with measurements I, II, and III.

TABLE 6

SETTING EXPANSION OF INVESTMENTS

	Per Cent Expansion		Per Cent Expansion
Manufacturer's value for E.....	0.26	*From this work, model A only:*	
From the National Bureau of Standards for E.....	0.21	Horizontal expansion of cast for E..	0.14
		Vertical expansion of cast for E....	0.69
From Earnshaw's paper (ref. 2) for E..	0.62	Horizontal expansion of cast for T.	0.01
From Osborne and Skinner (ref. 1)....	1.10	Vertical expansion of cast for T...	0.64

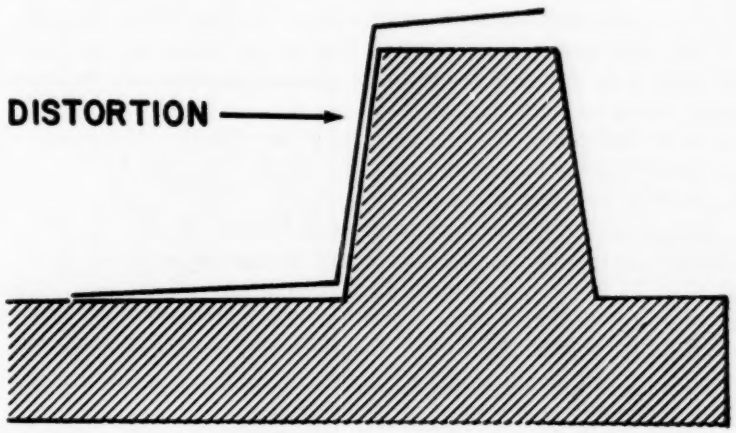

FIG. 6.—The general form of distortion of investment casts in the *standard position;* the changes are greatly exaggerated.

If the base is thicker, as in category SP2, the increased bulk may increase the lateral thrust, and a decrease in vertical expansion (free) occurs. Some of the apparent vertical increase was due to a change in the base—that is, a form of buckling occurred. The peripheral sections of the model tended to rise in relation to the base (line SP in Table 5). A diagrammatic representation is shown in Figure 6. A base about $\frac{1}{2}$ inch in thickness decreased such buckling and so decreased apparent vertical changes.

The stiffness of the jell within the range of dilution that may inadvertently occur with loss of water during continued use of reversible hydrocolloid does not change the amount of expansion; the values in the lines SPRT/A and SPRT/B in Table 5 are not significantly different.

The rotation of the model through 90°, so that the equivalent of an anterior segment is duplicated, does not alter the expansion. The values in the line SPRT in Table 5 show some tendency for a decrease, in contrast to line SP, but such a change is not statistically supported.

When the three anatomical aspects of the model are separated from the container by equal amounts of hydrocolloid, little difference from the standard position in degree of expansion in the vertical direction occurs (line CC in Table 5). However, with its narrow width, the model does not resemble oral structures. If the model resembled a cast, the vertical increase due to changes in the base might occur around the entire periphery.

When the critical dimensions—in this experiment, vertical and horizontal (or axial and lateral)—are confined at each end, the uniformity of increase in length is noted (line SPO, Table 5). The effect of doubling the size of the base, as well as confining the critical dimensions in colloid (line SPO2, Table 5), produces a cast in which the two dimensions have increased in a like manner.

The influence on dimensional changes of the shape, size, and relationship of parts in any assembly containing setting investment has recently come under scrutiny in other fields. In inlay casting work, the variables influencing setting expansion associated with geometry have been described[3, 4] and apparently are more important in relation to accuracy than the setting expansion of the investment. Similarly, Ady and Mahler[5] show that manipulative variables, such as spatulation and water-powder ratio, are secondary to the nature of the restraint upon setting expansion in determining the real change within an investment mold.

A design for duplicating flasks suggests itself: the direction of free expansion could be made parallel to the base. The cast to be duplicated would be trimmed until the thinnest possible base was obtained; then a plate similar in contour to a cast and roughly equivalent to the height of teeth could be fixed to the base. This assembly would then be placed with the posterior face of the cast upon the base of the duplicating flask (Fig. 7). Hydrocolloid would be poured in and cooled. The flask base would be removed, to reveal the posterior face of the cast and plate; if the plate were then removed, the cast could be slid back to free the teeth, and the cast then swung out of the flask. Investment would then be vibrated in. Some expansion would occur and would tend to be uniform in amount in critical regions.

Such a design of flask would produce a cast with a base at least $\frac{1}{2}$ inch thick and with less buckling (if it does occur in casts). The increase in length of the teeth would be commensurate with increases in other directions.

One clinical observation of some consequence is possible; frequently incisal and occlusal rests and accompanying clasps do not seat fully when the cast framework is tried in the mouth. Very frequently, pseudo-orthodontic treatment is required. In present laboratory procedures the teeth on the investment cast may be increased in length about ten times as much as horizontal or occlusal relations. The suggested design of duplicating flask would obviate this discrepancy.

It was concluded that the amount of expansion that occurs in investment casts is determined by the interaction of the setting expansion of the investment with the geometry of the system; the value for setting expansion, as determined by laboratory tests using strips on troughs of mercury, samples in lined casting rings, or cylinders

surrounded by hydrocolloid, will not necessarily show the increase in size of any part of a partial denture investment cast. Free expansion will cause an increase roughly ten times that of the increase in the dimension of the investment cast that is confined at either end of that dimension by hydrocolloid in the duplicating flask.

Distortion of the base of investment casts used in these experiments occurred. A minimum thickness of at least ½ inch might be recommended to minimize this warpage, if the warpage does occur in dental casts. The base of an investment cast, therefore, should not be as thin as possible but rather thicker than may be necessary for general handling qualities.

A design of duplicating flask which would tend to equalize the increase in critical

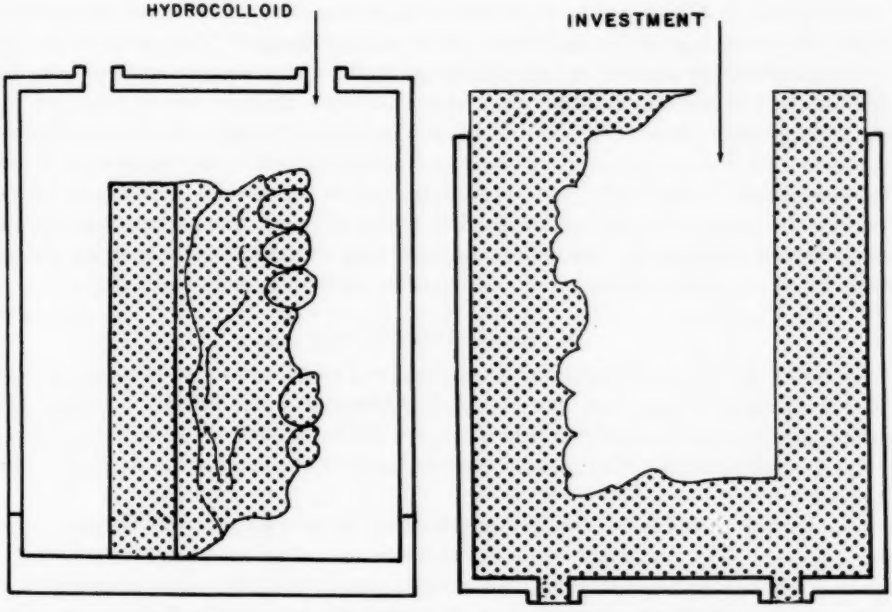

FIG. 7.—Diagrams, showing the experimental design of flask suggested

dimensions, due to setting expansion, has been described; such a flask should be made and tested in the future.

The lack of fit of occlusal and incisal rests in cast partial dentures when initially inserted has been explained as an undue increase in length of teeth in the investment cast because they lie in a dimension in which the investment is free to expand.

SUMMARY

Six investments were used to duplicate Invar models that represented a cross-section of a mandibular or maxillary cast. The investment casts were measured in two dimensions—parallel to the occlusal plane and parallel to the axis of the teeth. Marked differences in the increase in these two dimensions of the casts existed, and the increase due to setting expansion was about ten times greater in the vertical or axial direction when conventional duplicating flasks were used.

The amount of increase in the vertical or horizontal (occlusal) directions was not clearly related to a test where an asbestos-lined casting ring and dial gauge were used. The value of such laboratory tests in predicting the real expansion of some part of an investment cast was questioned. The geometry of the specimen undergoing setting expansion and the surrounding or confining medium influenced the amount of expansion.

A design for a flask in which equal expansion in critical dimensions could be obtained was described, and the castings made upon such casts are described in Part II of this paper. The frequent lack of fit of incisal and occlusal rests in cast metal partials was partly explained by the distortions during duplication of the master model.

II. THE CASTING PROCESS

Distortions or inaccuracy in fit of the final casting after the investment model is made are introduced by (*a*) distortions in the wax pattern, (*b*) distortions in the investment mold, (*c*) changes in the solidifying metal and subsequent variations due to recovery and alterations in stress distribution, and (*d*) changes due to polishing. In the fabrication of partial dentures the application and fastening of the wax to the investment cast removes one source of warpage; but all distortions or inaccuracies in the investment cast are faithfully reproduced. A method to improve the accuracy in reproduction of dimensions in the cast has been described (Part I). The changes, apart from those created by polishing, that follow the imbedding of the wax pattern on its investment model in a casting flask, were investigated by measurement of the castings.

MATERIALS AND METHODS

After the investment models had been measured (see Part I) a wax pattern was built up using 22-gauge sheet wax;* in each pattern the whole occlusal or horizontal surface of the model was covered with a uniform thickness (one or two sheets as specified). A variety of designs for sprues were tried and will be described in relation to the tables.

Gold-casting procedure.—The investment model with its pattern was fastened with wax to a base plate. A casting flask 9 cm. in diameter and 6 cm. in height was luted to the base plate. A thin asbestos liner was used inside the flask or casting ring and was positioned with the ends of the liner flush with both ends of the flask. A crucible-former of acrylic resin was attached to the sprue or sprues, so that the top of the crucible-former was flush with the open end of the flask. A water-to-powder ratio of 0.27 was used for the investing mixture, which was vibrated in; after 2 hours the assembly was prepared for the burn-out.

The flask was placed, sprue upward, in an electric furnace at room temperature. The temperature of the oven was raised to 700° C. for 1½ hours and maintained at this temperature for 1 hour.

The gold alloy was preheated in the electric furnace on the casting machine,† and at the appropriate time the casting ring was transferred from the oven and the casting rapidly completed.

The flask was immediately removed from the machine and placed in cool water. The

* Kerr Casting Pink Wax sheets.

† Thermotrol casting machine and oven supplied by J. F. Jelenko and Co.

casting was removed, cleaned by hand, pickled in hydrochloric acid, then sandblasted free of investment.

Cobalt-chromium procedure.—The pattern on the investment model with attached wax sprues and crucible, was painted with a small artist's brush with the investing mix (water-to-powder ratio 0.28).* When this mix had set, this assembly was held in a casting ring (9 cm. diameter, 6 cm. height) and more investing mix vibrated in. The sprue and crucible-former were positioned in the center of the flask.

The investment in the casting ring was allowed to set, usually overnight. Before heating in an electric furnace,† the investment and ring were placed in water. The furnace was brought from room temperature to 600° C. and held for 3 hours. The metal ingot‡ was then placed in the electric furnace on the casting machine§ and heated. As soon as the metal had reached a mushy consistency (tested with a stainless-steel probe), the flask was transferred to the machine and the casting completed. The orientation of the mold space was such that the longest part of the casting was in the vertical plane. The investment flask and casting were set aside and allowed to cool to room temperature. The castings were cleaned by hand and sandblasted prior to measurement.

Measurements.—The cleaned castings in both gold and cobalt-chromium alloys were measured in the as-cast condition (but cleaned and sandblasted) with the sprues attached (condition called *w.s.*). Then the sprues were cut off by carborundum disks in a dental handpiece. The casting was then remeasured (*w.t.s.*). After the sprues had been removed, the gold castings were annealed at 700° C. for 10 minutes and quenched before a third series of measurements (*ann.*) was made.

The system of measurement and averaging was similar to that outlined in Part I for the investment models. Six places on the "mucosal" surface of the casting and a horizontal distance between indentations were measured with the dial gauge and microscope comparator, respectively. The values used in the statistical analysis were again I, II, III, representing percentage errors in vertical reproduction, and IV, in horizontal reproduction. Each value in the raw or analyzed data represented an average of twelve readings, and in each category three or more castings were used.

Statistical notes.—The variation among measurements of castings was at least four times that of a similar group of investment models. The mean square of the residual in the analysis of variance in the best series of castings was 0.11, whereas the best replication of investment casts gave a similar mean square of 0.03. This difficulty in replication reflects the greater variation in the casting process due to a lack of precise control of variables. Thus, even though some seventy castings were available, conclusions about each category in which four to six castings were used were not clear.

The percentage error in the casting, when related to the master model, may indicate the relative fit of the casting; this error, called here *total distortion,* came from two sources—the investment model (dealt with in Part I) and the casting process. The percentage errors, or *casting distortions,* due to the casting process alone were calcu-

* The instructions supplied with Ticonium materials and equipment were followed throughout the cobalt-chromium procedures.

† Ticonium preheating oven with Simplytrol controller.

‡ Ticonium alloy, ingots ¾ inch long.

§ Ticonium casting machine.

lated by subtracting the percentage error of each investment model from the percentage error of the related casting.

Duncan's test[6] was used extensively in this section in preference to the Student *t*-test. According to Duncan, the *t*-test is liable to a greater probability of error than the 1 or 5 per cent level given in the usual tables,* and the level of accuracy, as previously noted, was low. The shortest significant ranges (*s.s.r.*) are given where relevant. The number in parentheses refers to the relation of means; (2) indicates the *s.s.r.* for the difference between adjacent means, (3) for the difference between means when ranked in order, separated by one mean, (4) when ranked in order separated by two means, and so on.

In both parts of the investigation, 22 separate analyses were completed, using 80 investment models and 70 castings. The tables therefore have extensive backgrounds,

TABLE 7

TOTAL DISTORTION: GOLD

(Means of Percentage Errors)

INVESTMENTS	INVESTMENTS AND CONDITIONS				
	w.s.	w.t.s.	ann.	s.s.r.	
				2	3
A	1.27	0.90	0.55	0.46	0.49
D	0.24	0.51	0.83	0.38	0.40
E	0.11	−0.04	0.03	0.42	0.44

and significance in one analysis was not accepted unless another analysis showed the same factors again operating.

RESULTS

Table 7 shows the effects of three investments upon the total distortion of gold castings. Measurements were made in the three conditions with sprues attached (*w.s.*), without sprues (*w.t.s.*), and after annealing (*ann.*).

Table 8 is a summary of significantly different categories again with three investments; these data reflect the casting distortion of gold alloys only. The changes due to the setting expansion of the investment model have been removed. In Table 8, *a*, the investments and the condition of the casting are categorized. In Table 8, *b*, the measurements I, II, III (vertical), and IV (horizontal) are shown in relation to the investments A, D, and E.

Owing to the levels of significance associated with the number of replications in each category, it is not possible to break down further the averages in this analysis. The analysis behind Table 8 showed that the relationships between the investments and the measurements and between investments and conditions were significant (Table 8, *a*). A

* The errors will be omissions of significant differences rather than inclusions of non-significant differences.

lesser level of (5 per cent) significance was needed before the relationship between investments and measurements could be admitted.

The thermal expansion of investments A, D, and E (same batches) was reported by Osborne and Skinner[1] and in Table 9 it is shown in relation to the average change in two dimensions for the gold castings after the sprues had been removed (*w.t.s.*) and after annealing (*ann.*). No statistical limits for confidence are available with this table.

In Table 10 the casting distortion of cobalt-chromium alloys alone was analyzed,

TABLE 8

CASTING DISTORTION: GOLD

(Means of Percentage Errors)

a) INVESTMENTS AND CONDITIONS OF MEASUREMENT

INVESTMENTS	CONDITIONS				
	w.s.	w.t.s.	ann.	s.s.r.	
				2	3
A	0.25	−0.02	−0.33	0.46	0.49
D	−0.83	−0.62	−0.41	0.38	0.40
E	−0.46	−0.56	−0.55	0.42	0.44

b) INVESTMENTS AND MEASUREMENTS

INVESTMENTS	MEASUREMENTS				s.s.r.		
	I	II	III	IV	2	3	4
A	−0.35	−0.15	−0.37	−0.04	0.53	0.36	0.58
D	−0.99	−0.84	−0.51	−0.12	0.44	0.46	0.48
E	−0.72	−0.62	−0.63	−0.11	0.48	0.51	0.52

TABLE 9

CASTING DISTORTION RELATED TO THERMAL EXPANSION: GOLD ALLOY

(Means of Percentage Errors)

INVESTMENTS	DIMENSION MEASURED	CONDITION		THERMAL EXPANSION (OSBORNE-SKINNER)
		w.t.s.	ann.	
A	Vertical	−0.05	−0.39	0.95
	Horizontal	+0.23	−0.15	
D	Vertical	−0.80	−0.47	0.52
	Horizontal	−0.07	−0.19	
E	Vertical	−0.73	−0.66	1.15
	Horizontal	−0.04	−0.20	

but two models of slightly different shape were used. Horizontal changes for the models were not significantly different and were not noted.

Table 11 shows the change that occurred with various sprue forms before and after the sprues were removed. To understand the form of sprueing used, the casting is to be imagined as seated on the cast, and the following forms can be described:

Side sprue.—A conical sprue directed parallel to the occlusal surface was attached to the center of the posterior edge of the flat "interalveolar" plate.

Top forked sprues.—Two cylindrical feeders (diameter 25 mm.) in the vertical plane were attached to the occlusal sections on the inner portions of the ridges. Reservoirs were placed close to the termination of the feeder on the casting proper.

TABLE 10

CASTING DISTORTION: COBALT-CHROMIUM ALLOY: THE EFFECTS OF MODEL SHAPE

(Means of Percentage Errors)

	Av. Vertical Change		Av. Vertical Change
Model A.....	−1.07	Model B.....	−0.72

TABLE 11*

CASTING DISTORTION: COBALT-CHROMIUM ALLOY

(Means of Percentage Errors)

FORM OF SPRUE OR CASTING	CONDITION OF CASTING		S.S.R.
	W.S.	W.T.S.	
Side sprue.....	−0.54	−0.30	0.24
Top forked sprues.....	−0.94	−0.24	0.27
Side occlusal sprue.....	−0.73	−0.18	0.31
Side sprue, clasps.....	−0.29	−0.18	0.31
Side sprue, double thickness.....	−0.94	−0.30	0.24

* Form of casting and condition when measured; vertical change in model B only.

Side occlusal sprue.—This was a conical form of sprue with a large reservoir or ball at the end. This sprue was vertical, and a side auxiliary sprue or feeder above the ball led sideways (horizontally) to the edge at the center of the casting.

Side sprue with clasps and side sprue with double thickness.—These were variants of the side-sprued form. The clasps were attached mesially and distally on the occlusal section of the ridges. In one series, two sheets of wax were used to create a "double-thickness" casting.

In Table 12 all forms of sprueing are combined for master model B, and the means for vertical measurements and change in conditions are given in contrast with similar figures for gold alloys cast, using investment E.

The results of these analyses and others led to a hypothesis about this particular casting in cobalt-chromium alloy, and the curved side sprue was evolved. Table 13 compares the total distortion of the various side-sprue types.

Table 14 indicates the direction and amount of change that occur with three different sprue forms when the sprues are removed. The same materials and techniques were used for casting all these specimens.

DISCUSSION

Gold castings and investments.—When the sprues were cut on the gold castings, the change that occurred in the measured dimensions was not significant; in Table 7 the as-cast state (*w.s.*) is compared with the casting without sprues (*w.t.s.*). A significant change in dimension occured between the as-cast and the annealed state (*w.s.* to *ann.*)

TABLE 12*

CASTING DISTORTION

(Means of Percentage Errors)

	MEASUREMENTS			CONDITIONS		
	I	II	III	w.s.	w.t.s.	ann.
Cobalt-chromium.....	−0.49	−0.85	−0.79	−0.82	−0.60	.....
Gold, investment E....	−0.72	−0.62	−0.63	−0.58	−0.73	−0.66

* Vertical changes in model B only.

TABLE 13*

TOTAL DISTORTION: COBALT-CHROMIUM ALLOY

(Means of Percentage Errors)

SPRUEING	MEASUREMENTS	
	Vertical	Horizontal
Side sprue.....	0.53	−0.82
Side occlusal sprue.....	0.43	−0.72
Curved side sprue.....	−0.32	0.57

* Confidence limit for difference of two means = 0.80.

TABLE 14*

STRESS RELEASE WHEN SPRUES WERE REMOVED: COBALT-CHROMIUM ALLOYS

(Means of Differences in Percentage Errors)

SPRUEING	MEASUREMENTS		
	I	II	III
Side sprue.....	0.35	0.25	0.06
Side occlusal sprue.....	0.05	0.10	0.14
Curved side sprue.....	−0.29	−0.61	−0.26

* Confidence limit for difference of two means = 0.14.

when investments A and D *but not* E were used. The change in dimension *after* casting, therefore, is related to the investment used.

According to traditional theory, the shrinkage of metal during solidification should be compensated for by the setting and thermal expansion of the investment. On the basis of Table 7 and some of the facts that follow, it is suggested that the crushing strength of the *hot* investment will determine the changes or distortions rather than the setting and thermal expansion. The strength of the investment model at the time of metal solidification will determine whether the shape of the casting is maintained as the metal solidifies and contracts upon the investment cast. In Table 7, investments A and D did maintain the shape of the casting in part, and this resistance to solidification shrinkage was represented by residual stress in the casting. Hence, after annealing, castings made in investments A and D changed shape. The casting made with investment E, where little resistance was offered to solidification shrinkage (postulated, not proved), did not change shape on annealing. The changes after casting apparently were not related to the compressive strength before the thermal cycle; A and D had lower strengths than E after 1 hour of set.[1] Residual stress and changes in its distribution may be a greater influence on fit or accuracy than the setting or thermal expansion of investment.

The distortion due to the thermal cycle alone is shown in Table 8. Castings made in investments A and D changed in passing from the as-cast (*w.s.*) to the annealed (*ann.*) state, whereas those made in E remained noticeably steady. It is curious that the varying changes with the three investments produced finished castings with roughly the same discrepancy in the annealed state. The difference in the annealed state of the castings shown in Table 7, and those of Table 8, *a*, was due to the effects of setting expansion upon the dimensions of the investment cast (Part I).

In Tables 7 and 8 the shape cast and the alloy used were constant. But the path by which the casting arrived at its final state seemed to depend on some property of the hot investment, and we believe that it was the hot strength of the investment. Similar findings were shown by other investments not included in this paper.

Different investments produced different changes in various directions. In looking across Table 8, *b*, the pattern of discrepancy differs in all three investments. Unfortunately, insufficient data were available to explore fully this implication. The change in the castings is shown diagrammatically in Figure 8. There seems to be rotation in the gold castings rather than buckling, and an attempt was made to demonstrate this statistically. While a tendency could be detected, the level of significance was low.

The conclusion reached is, therefore, that the setting and thermal expansions of investments do not directly indicate the fit of a partial denture casting. Table 9 illustrates this conclusion. Not only are there variables associated with differences between investments, but the casting itself changes in a different way with each investment.

By considering averages instead of individual readings, general conclusions only may be reached, and consequently no specific attributes of any investment can be given.

Cobalt-chromium castings.—The investigation in this section was restricted to the influence of shape and geometry upon accuracy. From Table 10 it is deduced that model shape or pattern shape will affect the amount of change. Figure 1 in Part I showed the different shape of the Invar models used. This geometrical influence upon

distortion indicates a certain danger in extrapolating these data to cast partial dentures of a clinical design. The conclusions reached will be general rather than specific.

Table 11 shows the geometrical influence of the *whole* casting, that is, sprues, reservoirs, and dental appliance. The various shapes and style of sprue were noted before. In Table 11 a change from the as-cast to the without-sprue state indicates a change in the distribution of residual stress. The categories *side sprue* and *side sprue with clasps* show the least change in shape. It was deduced, therefore, that side-sprueing produced the most stable stress distribution. Why the inclusion of clasps did not *increase* the changes or distortion is uncertain; their presence in a critical area of distortion (as shown by changes in measurement I) should alter the distribution of residual stress in the cooled casting because the clasps, by being locked in the investment, hold back shrinking parts. When the casting undergoes recovery or redistribution of residual stress, a noticeable change might be expected. The alternative suggestion is that the

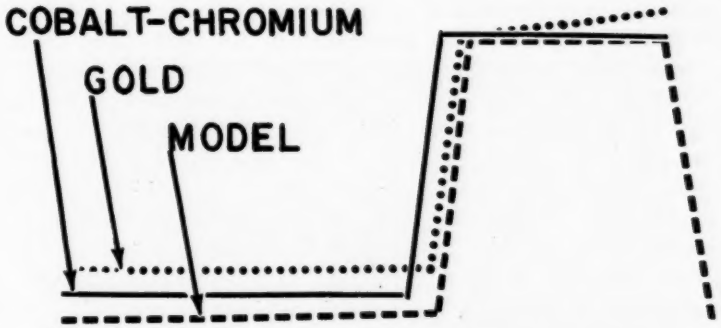

FIG. 8.—A diagram, showing in an exaggerated fashion the distortion due to the casting process only for model A. The measurements I, II, III, and IV described in the text were used; the casting between the points of measurement was not necessarily straight or flat as shown.

thermal gradient (hot-spot) in this region was such that stress was relieved during solidification; hot-spots will be mentioned later. No decrease in warpage occurred when the pattern was of double thickness; perhaps the increased strength of the casting, in this case, prevented stress relief during cooling.

To contrast gold and cobalt-chromium alloys, Table 12 is included. Investments E and T, which are used for cobalt-chromium castings, supposedly differ only in oxalate content.[2] In gold alloys the order of change in measurements is as follows: I is greater than II and III. In the cobalt-chromium, III is greater than II, and III and II are greater than I (Fig. 8).

Hence the different alloys produced a different amount of distortion in different parts. The effects of solidification shrinkage or residual stress distribution were determined by the temperature and width of the melting range, as well as by the strength of the cooling alloy. The greater hot strength expected in the cobalt-chromium alloys suggests that solidification contraction in the cobalt-chromium alloys crushes or distorts the investment cast more than a gold alloy would, and hence the *residual* stress would be less. Alternatively, recovery may not occur at 700° C. in castings of cobalt-

chromium. It would follow, and it does, that annealing cobalt-chromium alloys produces little change in shape; consequently, values for the annealed state in castings of cobalt-chromium alloys are not shown.

On the other hand, the geometry of the casting leads to a certain pattern of stress which is not relieved or redistributed until the geometry is altered. When the sprue is removed, the dental casting proper changes shape. The degree of this change depends on the location and number of sprues.

At this stage in the investigation it was decided that if an investment model which had expanded evenly in all critical dimensions were available, a better-fitting casting should result if changes due to residual stress were diminished. The changes in gold-alloy castings due to residual stress, as has been shown, compensated in part for a distortion in the investment model, and thus residual stress would have to be minimized if a more accurate model were used. Authorities on residual stress and investment casting were consulted.

Investment casting and residual stress.—Thermal gradients; the solidification rate at any site in the mold depends on the temperature in that place during cooling, and, in referring to the temperature difference in various parts of the mold, the term "temperature gradient" is used. Non-uniform cooling produces mismatch in the casting, and either a flaw forms, or stress is generated. If the cooling is rapid, then the stress will not be relieved by flow of the metal, and hence there will be residual stress. In the design of centrifugal castings industrially, hot-spots are avoided.[7] In many dental-casting procedures, reservoirs are used; these do not operate necessarily as a source of molten metal to fill in solidification shrinkage, but rather as disturbances of the thermal gradient or hot-spots. The bulk of metal in the reservoir generates a hot-spot, and cooling thereabouts is less rapid. With the almost instantaneous solidification in investment molds, reservoirs may not be necessary if the sprue is of sufficient size. Any effect of the large sprue on thermal gradients will be dispersed over a wide region, and hence more uniform cooling rates will result.

The freezing range, the rate of flow, the temperature of the mold, and the metal will also affect the development of residual stress, and these differences are partly shown in the data.

Constraint upon shrinkage; restraint by the surrounding investment in the solidifying metal will also determine stress generation. Restraint in the dental mold arises from the shape of the pattern, the form of the sprues, reservoirs, feeders, and so on, and from the strength of the surrounding investment at the temperature of casting. Industrially "the best way to avoid distortion is to design the casting properly."[8] In dental work, only the design of the sprues can be readily altered.

Flow of metal; the pattern of the flow of the metal is partly determined by the sprue design. Instantaneous filling of the mold is desired, and any disturbance to flow may produce a wave pattern in the casting itself.[9, 10] The thermal gradient in the casting is altered by disturbances in flow. Free, unimpeded flow without turbulence or interruption is desirable. The nature of the flow is determined by the shape, size, and number of sprues, the shape and volume of the mold cavity, the junction between mold and sprue, and the casting machine.

Experimental design.—The following were selected for trial in this investigation.

1. Sprue form and number: A tapered sprue was selected. As molten metal passes

down a tube, the stream tends to narrow. The driving force falls (decrease in pushing due to metal), and the viscosity increases with cooling. If the stream becomes detached from the walls of the sprue, turbulence and gas inclusion occur. Therefore, the sprue should be tapered to conform with the expected shape of the stream of hot metal.

2. The exertion of a steady, even pull upon all regions of the casting during mold-filling seems desirable. In fact, one observation made was that casting failures can be attributed to a lack of orientation of the casting; in every successful casting the flask was placed in the machine with the lateral axis of the casting in the vertical plane. To insure casting the major divergences in pattern (or mold space), the sprue axis was placed to the right of this axis. Metal would be pulled into the mold space by the force of inertia during rotation of the casting arm (toward the left in relation to the sprue axis).

3. One large sprue was used. The thermal gradients are less disturbed. Restraint upon shrinkage is less when no "reinforcements" exist. It was noted that side sprues always caused less total distortion.

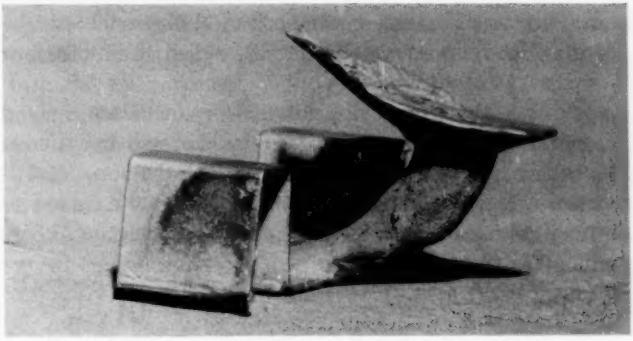

FIG. 9.—The experimental design for sprueing

4. As cooling occurs from the outside in, a central and "side-on" position of the sprue on the casting is recommended,[7] to prevent "bellying"; this distortion arises in long, flat sections and occurs in the radial plane at right angles to the sprue direction or axis. This influence may be associated only with the form of casting used in this study.

5. Large, sudden increase in volume in mold space, especially where the sprue joins the casting, should be avoided.[8–10] Cooling occurs as the molten metal expands into the increased space, and solidification is encouraged. Thus the sprue was attached to the central plate, and no reservoir or widening of the sprue near the casting proper was used.

The design of the final series of castings was based on the preceding. A tapered, *curved side sprue* was attached centrally (Fig. 9). No reservoir was contemplated. The curve in the sprue placed the casting away from the center of the mold. The drag upon the metal due to inertia during rotation and the outward driving force due to the centrifugal action would give a resultant pull on the incoming metal at an angle to the axis of the casting arm. The casting itself was at 20°–30° to the axis of the casting arm; it has been estimated that an angle of 15° would be more suitable.[11]

The casting machine was checked completely and very carefully balanced for each flask; all retaining bolts were tightened, and vibration of the machine was negligible. Vibration of the casting machine has been related to distortion.[9, 10]

Some consideration as to the applicability of industrial recommendations is needed. Industrially, an accuracy of ±0.005 inch per inch is expected from castings about 3 inches in size. The engineering precision expected from dental castings in this study was ±0.06–0.18 mms., and a range of ±0.03 mm. was apparently easily obtained. Fit, by engineering standards, remained poor. The shape of the casting caused this lack of fit. Because a flat piece was inclosed between walls with a divergence of 5°, a small increase in the central portion would prevent the casting from seating. It is estimated that the percentage error which would allow seating would be less than 0.05 per cent. On the models used, this possible error represents approximately 0.05 per cent of 30 mm., that is, ±0.015 mm. or ±0.0006 inch. Such precision could scarcely be expected. Precision in casting a bar in cobalt-chromium has been given[2] as ±0.2 per cent.

Teeth in a non-pathological state will move from 0.02 (molars) to 0.14 mm. (incisors). Comfortable fit in the span between two molars would therefore be about ±0.01 mm., and such accuracy was available in the horizontal dimension (see below). But fit is not possible if the distortions due to changes in residual stress distribution warp the casting.

In Table 13 a review of the accuracy of the side-sprue variants is given. The curved side sprue produced a different pattern of stress release or redistribution and of dimension reproduction. Vertical changes were diminished, and the horizontal dimension was increased rather than decreased. The dimensional change in the curved side sprue produced the best-fitting casting in the entire study and its dimensional changes are given in the accompanying table.

	I	II	III	IV
Error per cent	0.23	—0.23	—0.39	0.00
mm. difference	+0.03	—0.03	—0.05	0.00

If the averages were taken for this series of castings, a false conclusion might arise. In the four castings, two increased and two decreased in dimension relative to the master model; the average would indicate excellent reproduction of accuracy. In the other castings, the errors were generally of the same sign. Apparently, other variables need control, such as the quantity of the metal in the sprue and in the crucible.

However, Table 13 illustrates the powerful effects of sprue design. Changes due to stress redistribution still occurred with the *curved side sprue,* and Table 14 is included to show the nature of this change in all side-sprue variants. The *side occlusal sprue* presumably changes least when the sprues are removed because the ball or large reservoir acts as a "hot-spot," and stresses are distributed in a more stable fashion (if such is possible). Nevertheless, the fit or accuracy in *side occlusal sprues* did not seem as satisfactory as the curved side sprue, perhaps because of the slightly larger horizontal increase. An attempt was made to relate this discussion with previous work. The setting and thermal expansion of the investments (same batches used) were determined,[6] and little relationship was found. Likewise, other recent investigations were compared,[1, 12] and it appears that the geometry of the casting and sprues has more to do

with fit of castings than does the thermal and setting expansions of the investment and the casting shrinkage of the metal. The difficulties of relating the setting and thermal expansions of investment to fit were described in 1934 in relation to inlays.[13] In 1942 and 1947 it was indicated,[13, 14] by work at the National Bureau of Standards, that the shrinkage of metal alloys usually referred to in the literature is in relation to a *specific shape and size*. The hot strength of some investment has been described[13] but was not related to distortion or changes during solidification shrinkage.

The model in this investigation resembled part of a partial denture, and it has been indicated that extrapolation of these data to clinical partial dentures is unwise except when principles are discussed. Some of the problems associated with the accuracy of large castings have been defined rather than solved.

It was concluded that the importance of residual stress, of its generation by sprue form and size, and the effects of the shape of the casting were shown by this investigation.

When gold-alloy castings were annealed, their shape changed, and the distortion in relation to the master cast decreased. The amount of such change was related to the investment used to make the cast and in filling the casting ring. However, the laboratory tests for setting and thermal expansions of these investments failed to show a relationship with the measurement of the castings. In fact, parts of the castings in themselves differed in amount of change due to the thermal processes associated with casting; changes in the axial or vertical direction (parallel to long axis of the teeth) were greater (ten to twenty times) than changes horizontally. As an explanation, the interaction of solidification contraction and hot strength of investment is suggested. The fact that cobalt-chromium alloys changed less during the removal of the sprues and scarcely at all on annealing was attributed to the greater hot strength or "crushing power" during solidification of these alloys, in contrast to gold alloys. The latter changed significantly in dimension during annealing.

The geometry (shape and size) of the casting was investigated with the cobalt-chromium alloy and technique. It was shown that the shape and number of sprues affected the fit of the casting.

Industrial science was investigated to ascertain current practice in casting of metals. However, it was found that the degree of accuracy or fit aimed at in dental castings and obtained in this study was about two to six times more "precise" than engineering criteria. The general conclusion reached about sprueing was that the effect of sprue size and form upon dimensional changes should be minimized because accurate casts were available (see Part I). The effects of sprue design were attributed to disturbances in thermal gradients and thus to stress generation and distribution.

On the basis of the conclusions reached, a sprue design was evolved. The curved side sprue produced the best single casting in the seventy made during this investigation. The change in stress distribution during the removal of this form of sprue altered the direction but not the degree of change, in contrast to similar side-sprued forms. Somewhere between the varieties of sprue forms used would be a design producing more accurate casting for this particular shape. In general terms the following could be recommended:

1. Single sprues are preferred to multiple feeders.

2. Reservoirs may not be necessary and may be undesirable; a large sprue would be preferred.
3. A tapered sprue attached to a region of similar size should permit better flow.
4. Central location of the sprue is recommended.
5. Vibration during casting in the casting machine may cause inaccuracy.

The effects of sprue designs were demonstrated upon a certain shape of casting. The relation of this shape to dental castings cannot be clear, because shape itself seems to be the major influence on accuracy of reproduction.

SUMMARY

Two master models made of Invar were used, and castings of a gold alloy and a cobalt-chromium alloy were made. Three different investments were used with the gold-casting process. From measurement of approximately seventy castings and a statistical analysis of these data, several general conclusions about the effects of the thermal process associated with dental casting were made.

The change in shape when the sprues were removed from castings was due to a change in the distribution of residual stress. Various influences, such as sprue shape, size, and direction in relation to the dental casting, were discussed, as well as the probable effects of differing hot strength of investments and the nature of the alloys used.

The shape of the casting itself, including sprues, reservoirs, and amount left in the crucible, would seem to determine the accuracy of large castings.

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A Study of Tooth Development by Tetracycline-induced Fluorescence

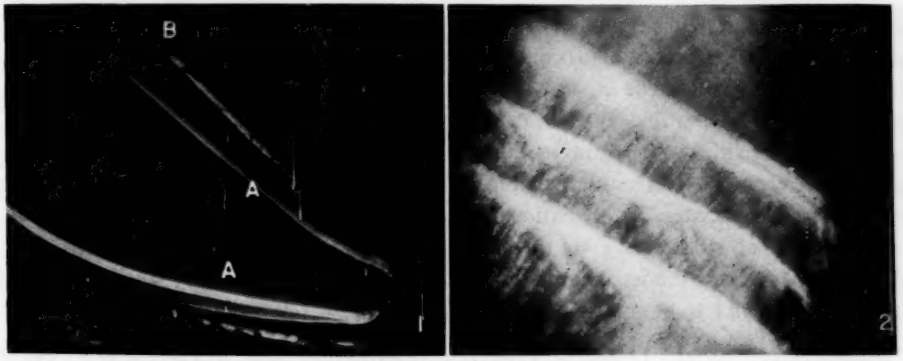
PHILIP J. BOYNE and CLARENCE W. MILLER

Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland

Recent studies have demonstrated the deposition of fluorescent material in areas of new bone formation following the parenteral administration of tetracycline antibiotics (R. A. Milch, D. P. Rall, and J. E. Tobie, *J. Bone Joint Surg.*, **40A**:897, 1958). The phenomenon of skeletal localization of these antibiotics has led to speculation regarding the possibility of their application to other fields of investigation. In order to determine whether tetracyclines could be utilized in the study of tooth and alveolar bone growth and development, a pilot observation was undertaken in eight mongrel dogs.

Single intravenous doses consisting of 10 mg/kg of oxytetracycline and chlortetracycline were given alternately at 10-day intervals to animals having morphologically developing cuspid teeth.

Examination of ground unstained histologic specimens with ultraviolet dark-field illumination revealed characteristic yellow fluorescent patterns within the developing dentin, resulting from



FIGS. 1 and 2.—Fig. 1: Ground undecalcified section of dog mandible photographed under ultraviolet light and illustrating three tetracycline-induced fluorescent bands in the root of the cuspid tooth (*A*), as well as in the periodontal bone (*B*). Dentinal and osseous tissue between the fluorescent patterns represent 10-day growth increments. (Mag. ×6.) Fig. 2: Area *A* from Fig. 1 photographed with ultraviolet illumination, illustrating the relationship of induced fluorescent bands to the dentinal tubules. The autofluorescence of the tubules can be easily distinguished from the induced fluorescence on the basis of color characteristics. (Mag. ×230.)

the administration of oxytetracycline, and deeper orange patterns following chlortetracycline injection. Variations in characteristics of the fluorescent bands made possible chronologic orientation of growth patterns in the developing teeth. In addition, characteristic fluorescent increments were produced in the adjacent periodontal bone (Figs. 1 and 2).

Inhibition and malformation of growing bone in the chick embryo following tetracycline injection has been reported (G. Bevelander, H. Nakahara, and G. K. Rolle, *Nature*, **184**:728, 1959). At the dosage level employed in our study, we were not able to detect any inhibition of tooth root development or calcification in the experimental animals as compared with similar teeth in the control dogs.

Tetracycline-induced fluorescence was found to have persisted in the teeth of dogs 12 months after administration of a single intravenous dose of the drug. Further experimentation is suggested to determine the clinical significance of this phenomenon.

The microscopy of tetracycline-induced fluorescence would appear to be an excellent means of chronologically orienting areas of dental calcified tissue growth and development.

The opinions or assertions contained in this article are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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